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(57) Abstract			
<p>This invention discloses high-affinity oligonucleotide ligands to complex tissue targets, specifically nucleic acid ligands having the ability to bind to complex tissue targets, and the methods for obtaining such ligands. Tissue targets comprise cells, subcellular components, aggregates or cells, collections of cells, and higher ordered structures. Specifically, nucleic acid ligands to red blood cells ghosts, glioblastomas, and lymphomas are described.</p>			

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Systematic Evolution of Ligands by Exponential Enrichment: TISSUE SELEX

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5 funded through the National Institutes of Health. The U.S. Government has certain
rights to this invention.

FIELD OF THE INVENTION

Described herein are methods for identifying and preparing nucleic acid
10 ligands to tissues. Tissues are described herein as a collection of macromolecules in a
heterogeneous environment. According to this definition, tissues encompass a single
cell type, a collection of cell types, an aggregate of cells or an aggregate of
macromolecules. The method utilized herein for identifying such nucleic acid ligands
is called SELEX, an acronym for Systematic Evolution of Ligands by EXponential
15 enrichment. Specifically disclosed herein are high-affinity nucleic acid ligands which
bind to various tissues.

BACKGROUND OF THE INVENTION

A method for the *in vitro* evolution of nucleic acid molecules with
20 highly specific binding to target molecules has been developed. This method,
Systematic Evolution of Ligands by EXponential enrichment, termed SELEX, is
described in United States Patent Application Serial No. 07/536,428, entitled
"Systematic Evolution of Ligands by Exponential Enrichment", now abandoned,
United States Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled
25 "Nucleic Acid Ligands", United States Patent Application Serial No. 07/931,473, filed
August 17, 1992, entitled "Nucleic Acid Ligands", now United States Patent No.
5,270,163 (see also PCT/US91/04078), each of which is herein specifically
incorporated by reference. Each of these applications, collectively referred to herein as
the SELEX Patent Applications, describes a fundamentally novel method for making a
30 nucleic acid ligand to any desired target molecule.

The SELEX method involves selection from a mixture of candidate
oligonucleotides and step-wise iterations of binding, partitioning and amplification,
using the same general selection scheme, to achieve virtually any desired criterion of

-2-

binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound
5 specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule.

10 The basic SELEX method has been modified to achieve a number of specific objectives. For example, United States Patent Application Serial No. 07/960,093, filed October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure", describes the use of SELEX in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics,
15 such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7,
20 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine", describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed Counter-SELEX. United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential
25 Enrichment: Solution SELEX", describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled "Methods of Producing Nucleic Acid Ligands" describes methods for obtaining improved nucleic acid ligands after SELEX has been performed.
30 United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled

-3-

"Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX", describes methods for covalently linking a ligand to its target.

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified nucleotides are described in United States Patent Application Serial No. 08/117,991, filed September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides", that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH₂), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029, filed June 22, 1994, entitled "Novel Method of Preparation of 2' Modified Pyrimidine by Intramolecular Nucleophilic Displacement", describes oligonucleotides containing various 2'-modified pyrimidines.

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX" and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX", respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

Without question, the SELEX process is very powerful. However, to date the process has been successfully demonstrated primarily with pure, simple

-4-

demonstration that complex targets are also compatible with the SELEX process. Tissue SELEX allows one to obtain nucleic acid ligands to multiple targets simultaneously, and is analogous to performing individual SELEX experiments on all the discrete components of a particular tissue.

5 It is desirable to be able to obtain nucleic acid ligands to complex tissue targets for various reasons. First, tissue SELEX can be useful to obtain nucleic acid ligands when a distinct target is unknown but a general mode of action of the desired ligand is suggested. Second, tissue SELEX can be useful when nucleic acid ligands are desired based on functional results. Since whole tissues or cells can be used in the
10 SELEX process, it is possible to select for nucleic acid ligands which produce a particular phenotype in the tissue or cell. Third, it can be desirable to obtain nucleic acid ligands to a complex tissue target when it is unclear which single target would be effective. It is also useful to obtain nucleic acid ligands to a complex tissue target if the purified target is unavailable or unstable in its purified form (i.e., a membrane
15 protein). Tissue SELEX allows the potential generation of ligands to previously unknown targets, and may rival monoclonal antibodies as reagents for research, diagnostics and therapeutics.

BRIEF SUMMARY OF THE INVENTION

20 The present invention includes methods of identifying and producing nucleic acid ligands to complex targets such as tissues and the nucleic acid ligands so identified and produced. More particularly, nucleic acid ligands are provided that are capable of binding specifically to tissues which are macromolecules in a heterogeneous environment, such as whole cells or substructures thereof, aggregates of cells,
25 collections of cells, aggregates of macromolecules and the like.

 Further included in this invention is a method of identifying nucleic acid ligands to tissues comprising the steps of (a) preparing a candidate mixture of nucleic acids, (b) partitioning between members of said candidate mixture on the basis of affinity to tissue, and (c) amplifying the selected molecules to yield a mixture of
30 nucleic acids enriched for nucleic acid sequences with a relatively higher affinity for

-5-

binding to tissue. Also included are nucleic acid ligands identified according to such method.

Another embodiment of the invention includes methods wherein a negative selection is performed in order to perfect the discrimination between subtle differences of similar tissue types. In this embodiment, the resulting ligands are specific not only for a particular tissue type, but can discriminate between subtly different tissues of the same type. For example, this method can discriminate between normal and abnormal tissue types, between induced and uninduced tissue types, etc.

In another embodiment of the invention, a method is provided for identifying previously unknown or uncharacterized epitopes which are components of a larger unknown macromolecule, on the tissue target. The ligands that are evolved by the present invention are capable of binding to previously unknown epitopes and the macromolecule which comprises the unknown epitope can then be identified by standard methods. For example, ligands can be evolved to a previously unknown protein found in the context of a complex tissue target. The ligand of the invention can be used to purify the protein away from the tissue target by standard protein purification and identification methods. These standard methods include affinity purification, microsequencing and cDNA databank searches. In this aspect, the newly identified epitopes which are components of a larger unknown macromolecule, such as new or previously uncharacterized proteins, are provided by the invention. These new epitopes and the macromolecule of which they are a component will be useful as diagnostic and therapeutic agents as well as the ligands that helped identify them.

More specifically, the present invention includes nucleic acid ligands to red blood cell ghosts, human tumor cell lines, such as a T-cell lymphoblast cell line, CEMss, and an adherent cell line, the glioma U-251, including those ligands listed in Tables 1 and 2. Also included are nucleic acid ligands to the above-described tissues that are substantially homologous to any of the given ligands and that have substantially the same ability to bind the above-described tissues. Further included in this invention are nucleic acid ligands to the above-described tissues that have substantially the same structural form as the ligands presented herein.

-6-

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of crosslinking a ligand to red blood cell ghosts [(c56t) (SEQ ID NO:4)] and nucleic acids of similar, but scrambled, sequences to red blood cell ghost membrane extracts. A distinct protein band is identified specifically by the ligand. Shown are a silver-stained 6% SDS gel and autoradiography of the same gel. Irradiations were performed with a hand-held transilluminator (254 nm) and samples were separated by gel electrophoresis under denaturing and reducing conditions. 1-0' irradiation c56t (SEQ ID NO:4); 2-5' irradiation c56t (SEQ ID NO:4); 3-0' irradiation scrambled oligo #1; 4-5' irradiation scrambled oligo #1; 5-0' irradiation scrambled oligo #2; 6-5' irradiation control oligo #2.

Figure 2 shows the photoaffinity crosslinking of the truncate ligand c56t to RBC ghosts. 10^7 ghosts were mixed with 1 nM of c56t and irradiated with a 254 nm hand-held transilluminator for 0 or 5 minutes. The irradiations were performed in the absence of cold competitor, with 10 μ M cold c56t (as a specific competitor) or 10 μ M cold c16t (as a non-specific competitor). The photoaffinity reactions demonstrate the high affinity and high specificity of the ligand-protein interaction. Shown are SDS-PAGE results under both reducing and non-reducing conditions (both conditions are denaturing). The doubling of the molecular weight of the crosslinked protein under non-reducing conditions suggests the target protein is a disulfide-linked hetero- or homo-dimer.

Figure 3 shows predicted secondary structures of six ligands which are the result of the RBC ghost SELEX. The six sequences are derived from the motif I (Figure 3A), II (Figure 3B) and III (Figure 3C) classes of sequences (two from each motif) are truncated to the smallest functional size, as based upon phylogenetic and computer folding algorithms. Base pairing within each molecule is predicted as based upon phylogenetic and computer folding algorithms. Notice that the two ligands from motif III share common primary and secondary structures, but are circularly permuted in relation to each other.

Figure 4 displays affinity photocrosslinking data for the motif I truncate c56t and the motif II truncate c16t. The nucleic acid ligands have been synthesized as shown in figure 1 with a six-carbon amino linker on the 5' end of each molecule. These 5' modified ligands were radiolabeled on their 3' end with alpha 32 P ddATP. The

-7-

amino linker was used to conjugate the ligands with the photocrosslinking reagent sulfo-HSAB. Approximately 5 nM ssDNA was mixed with 10 mM sulfo-HSAB in 200 mM triethylamine CO₂ (pH 9.5) and allowed to react 15 min. at room temperature and 15 min. at 37 degrees C. Approximately 10⁷ ghosts were mixed with 10 nM of each ligand conjugate in a volume of 15 μ l, incubated 30 min. at room temperature and irradiated for 100 pulses of a 308 nm excimer laser (175 mJ/pulse/cm²). The reaction was then mixed with an equal volume of 2X reducing SDS loading buffer and run on a 4-12% gradient SDS polyacrylamide gel. The gel was run, fixed and dried.

Radioactivity was detected by a Fuji phosphorimager. Shown are photocrosslinking reactions as described above for c56t and c16t, with two additional reactions for each ligand: one included the addition of 10 μ M cold, unconjugated c56t, the other the same concentration of c16t. These "cross competition" reactions demonstrate the high affinity and specificity of the photoaffinity crosslinking method.

Figure 5 shows the results of three rounds of selection for sequences within the final round rbc ghost SELEX pool that are specific for four distinct proteins on the rbc ghost membrane. The final round SELEX pool (round 25) was amplified using a "sense-strand" primer synthesized with a 5' six carbon amino linker group. The PCR product was radiolabeled with 3,000 Ci/mmol, 1.3 μ M (final) alpha ³²P dCTP ([cold dNTPS] = 100 μ M (final)). The sense strand was purified using denaturing PAGE and eluted from the gel matrix and precipitated. The phenyl azide compound sulfo-HSAB was conjugated to the pool and the nucleic acid conjugate used for photoaffinity crosslinking with the rbc ghosts. 10⁷ ghosts were irradiated with 10 nM pool conjugate in a volume of 15 μ l and in the presence of 12 μ M non-specific nucleic acid competitor (a 30 base random pool). The reaction was incubated for 30 min. at room temperature and irradiated for 100 pulses of a 308 nm excimer laser (175 mJ/pulse/cm²). The reaction was then mixed with an equal volume of 2X reducing SDS loading buffer and run on a 4-12% gradient SDS polyacrylamide gel. The gel contents were electroblotted to a nitrocellulose filter, the filter washed in water and dried. Radioactivity was detected by a Fuji phosphorimager. DNA sequences which showed crosslinking to four proteins (termed proteins 5, 6, 7, and 8) varying in apparent molecular weight from 170-30 kDa were isolated by sectioning the

-8-

nitrocellulose filter and placing the appropriate filter slices directly into PCR reactions for sequence amplification. The sequences were amplified for approximately 22 rounds, the sense strand purified, and the DNA reamplified for another 22 rounds. The resulting DNA was again purified, conjugated to sulfo-HSAB and used for the next
5 round of photoaffinity crosslinking. Figure 5 shows the photoaffinity crosslinking obtained after 3 rounds of the enrichment process described above.

DETAILED DESCRIPTION OF THE INVENTION

This application describes nucleic acid ligands to complex tissue targets
10 identified generally according to the method known as the SELEX process. As stated earlier, the SELEX technology is described in detail, and incorporated herein by reference, in the SELEX Patent Applications. This method, referred to as the Tissue SELEX process, incorporates complex targets in contrast to the more simple targets previously used in the SELEX process. Certain terms used to describe the invention
15 herein are defined as follows:

"SELEX" methodology refers to the combination of selection of nucleic acid ligands which interact with a target in a desirable manner, for example binding to a protein, with amplification of those selected nucleic acids as described in detail above and in the SELEX Patent Applications. Iterative cycling of the
20 selection/amplification steps allows selection of one or a small number of nucleic acids which interact most strongly with the target from a pool which contains a very large number of nucleic acids. Cycling of the selection/amplification procedure is continued until a selected goal is achieved.

"Tissue SELEX" methodology applies the SELEX methodology to
25 tissue targets. Tissue SELEX has several advantages. First, using Tissue SELEX one can obtain ligands to specific cell types in the absence of a defined understanding of the involved epitope. The epitope against which a ligand is evolved is usually a substructural component of a larger macromolecule. The ligands found by this method could also be useful in identifying new proteins or other new macromolecules on the
30 tissue target. The new proteins or other new macromolecules which comprise a newly identified epitope can be purified and characterized using standard procedures.

Second, ligands can be obtained to defined epitopes or macromolecules in the context of their physiologic cellular or membrane environment. Examples of various tissue targets can include a membrane protein on a whole cell, a plasma protein in plasma, a nuclear protein in the presence of whole nuclear extracts, etc. Third, it is possible to obtain ligands to tissues in a functionally altered phenotype, e.g., activated, migrating, etc. The ligands and the new macromolecules containing the ligand epitopes identified by this process may be useful as diagnostics or therapeutics. Fourth, Tissue SELEX is a powerful methodology which allows one to identify nucleic acid ligands that can mediate many different cell behaviors, such as apoptosis, anergy, differentiation, proliferation, etc., without prior knowledge of the identity of the specific tissue targets that control these changes. The sensitivity of the SELEX process may lead to the generation of oligonucleotides that recognize potentially every different epitope on the complex tissue target. Larger numbers of different sequence motifs are expected using the tissue SELEX process, as compared with simple-target SELEX, since it is believed that different motifs will recognize distinct epitopes on the complex tissue target. Some epitopes may lie within the same protein, but many will be directed to various proteins or other molecules on the tissue. Tissue SELEX can be done *in vivo* or *in vitro*.

Tissue SELEX allows one to work with a complete living "element" (a cell or bigger) that allow one to *phenotypically* screen for a target-ligand interaction that effects this "element." For example, one could screen an evolved, high affinity tissue SELEX pool using flow cytometry for sequences which bind a membrane protein and cause the cell to carry out a biochemical transformation which is measured by the flow instrument.

Tissue SELEX allows one to obtain nucleic acid ligands to multiple targets simultaneously. All independent binding sites on a very large macromolecular complex such as a tissue or cell should be potential targets for selection. In effect, this allows one to take a tissue and carry out numerous SELEX procedures on this tissue that is theoretically equivalent to individual SELEXes on all individual components of the particular tissue.

-10-

In one embodiment, a negative selection process (termed counter-SELEX) is employed to enhance the possibility that the ligands derived by tissue SELEX have precise specificity and affinity. In this embodiment, ligands are selected for a specific tissue and then a negative selection is done against a related
5 tissue which does not have certain characteristics for which the ligand is desired. The negative selection can be done against a similar cell line or cell type, different cells, normal tissue, plasma or blood, a non-specific antibody or other available ligand. An example of this negative selection would be to first select using a tumor cell target (such as a malignant melanoma) and then counterselect the resulting nucleic acids
10 against a similar cell type which is not tumorigenic (such as normal human melanocytes). Ligands that interact with both normal and neoplastic tissue will be removed by this negative selection and only those nucleic acid ligands that specifically bind the tumor cells will be identified (or retained). The resulting nucleic acid ligand would be specific for tumors. This technique will provide the ability to identify nucleic
15 acid ligands that can discriminate between two closely related targets, i.e., between a cancerous cell and an untransformed cell of the same tissue type. The negative selection can also be done *in vivo*. Using this method one can not only generate ligands to specific targets on complex tissue surfaces, but also be able to recognize the differences between normal and abnormal tissue of a particular type.

20 "SELEX Target" or "Target" refers to any compound upon which a nucleic acid can act in a predetermined desirable manner. A SELEX target molecule can be a protein, peptide, nucleic acid, carbohydrate, lipid, polysaccharide, glycoprotein, hormone, receptor, antigen, antibody, virus, pathogen, toxic substance, substrate, metabolite, transition state analog, cofactor, inhibitor, drug, dye, nutrient,
25 growth factor, cell, tissue, etc., without limitation. Virtually any chemical or biological effector would be a suitable SELEX target. Molecules of any size can serve as SELEX targets. A target can also be modified in certain ways to enhance the likelihood of an interaction between the target and the nucleic acid.

"Tissue target" or "Tissue" refers to a certain subset of the SELEX
30 targets described above. According to this definition, tissues are macromolecules in a heterogeneous environment. As used herein, tissue refers to a single cell type, a

- 11 -

collection of cell types, an aggregate of cells, or an aggregate of macromolecules.

This differs from simpler SELEX targets which are typically isolated soluble molecules, such as proteins. In the preferred embodiment, tissues are insoluble macromolecules which are orders of magnitude larger than simpler SELEX targets.

- 5 Tissues are complex targets made up of numerous macromolecules, each macromolecule having numerous potential epitopes. The different macromolecules which comprise the numerous epitopes can be proteins, lipids, carbohydrates, etc., or combinations thereof. Tissues are generally a physical array of macromolecules that can be either fluid or rigid, both in terms of structure and composition. Extracellular
- 10 matrix is an example of a more rigid tissue, both structurally and compositionally, while a membrane bilayer is more fluid in structure and composition. Tissues are generally not soluble and remain in solid phase, and thus partitioning can be accomplished relatively easily. Tissue includes, but is not limited to, an aggregate of cells usually of a particular kind together with their intercellular substance that form
- 15 one of the structural materials commonly used to denote the general cellular fabric of a given organ, e.g., kidney tissue, brain tissue. The four general classes of tissues are epithelial tissue, connective tissue, nerve tissue, and muscle tissue.

- Examples of tissues which fall within this definition include, but are not limited to, heterogeneous aggregates of macromolecules such as fibrin clots which are
- 20 acellular; homogeneous or heterogeneous aggregates of cells; higher ordered structures containing cells which have a specific function, such as organs, tumors, lymph nodes, arteries, etc.; and individual cells. Tissues or cells can be in their natural environment, isolated, or in tissue culture. The tissue can be intact or modified. The modification can include numerous changes such as transformation, transfection,
- 25 activation, and substructure isolation, e.g., cell membranes, cell nuclei, cell organelles, etc.

Sources of the tissue, cell or subcellular structures can be obtained from prokaryotes as well as eukaryotes. This includes human, animal, plant, bacterial, fungal and viral structures.

- 30 "Nucleic acid" means either DNA, RNA, single-stranded or double-stranded and any chemical modifications thereof. Modifications include, but

-12-

are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrogen bonding, electrostatic interaction, and fluxionality to the individual nucleic acid bases or to the nucleic acid as a whole. Such modifications include, but are not limited to, modified bases such as 2'-position sugar
5 modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at cytosine exocyclic amines, substitution of 5-bromo-uracil; backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping. Modifications that occur after each round of
10 amplification are also compatible with this invention. Post-amplification modifications can be reversibly or irreversibly added after each round of amplification. Virtually any modification of the nucleic acid is contemplated by this invention.

"Nucleic acid test mixture" or "nucleic acid candidate mixture" is a mixture of nucleic acids of differing, randomized sequence. The source of a "nucleic
15 acid test mixture" can be from naturally-occurring nucleic acids or fragments thereof, chemically synthesized nucleic acids, enzymatically synthesized nucleic acids or nucleic acids made by a combination of the foregoing techniques. In a preferred embodiment, each nucleic acid has fixed sequences surrounding a randomized region to facilitate the amplification process. The length of the randomized section of the
20 nucleic acid is generally between 8 and 250 nucleotides, preferably between 8 and 60 nucleotides.

"Nucleic acid ligand" is a nucleic acid which has been isolated from the nucleic acid candidate mixture that acts on a target in a desirable manner. Examples of actions on a target in a desirable manner include, but are not limited to binding of the
25 target, catalytically changing the target, reacting with the target in a way which modifies/alters the target or the functional activity of the target, covalently attaching to the target as in a suicide inhibitor, facilitating the reaction between the target and another molecule. In most, but not all, instances this desirable manner is binding to the target. In the most preferred embodiment, a nucleic acid ligand is a non-naturally
30 occurring nucleic acid ligand having a specific binding affinity for a tissue target molecule, such target molecule being a three dimensional chemical structure other than

-13-

a polynucleotide that binds to said nucleic acid ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said nucleic acid ligand is not a nucleic acid having the known physiological function of being bound by the target molecule. Nucleic acid ligand includes nucleic acid sequences that are substantially homologous to the nucleic acid ligands actually isolated by the Tissue SELEX procedures. By substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. In the past it has been shown that the sequence homologies of various nucleic acid ligands to a specific target shows that sequences with little or no primary homology may have substantially the same ability to bind the target. For these reasons, this invention also includes nucleic acid ligands that have substantially the same ability to bind a target as the nucleic acid ligands identified by the Tissue SELEX process. Substantially the same ability to bind a target means that the affinity is within a few orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence -- substantially homologous to those specifically described herein -- has substantially the same ability to bind a tissue target.

"Partitioning" means any process for separating nucleic acid ligands from the remainder of the unreacted nucleic acid candidate mixture. Partitioning can be accomplished by various methods known in the art. Filter binding, affinity chromatography, liquid-liquid partitioning, filtration, gel shift, density gradient centrifugation are all examples of suitable partitioning methods. Equilibrium partitioning methods can also be used as described in detail below. Since the tissue targets of the present invention are non-soluble, there are numerous simple partitioning methods which are well suited to this invention. The simple partitioning methods include any method for separating a solid from a liquid, such as, centrifugation with and without oils, membrane separations and simply washing the insoluble tissue target. The ligands can also be specifically eluted from the target with a specific antibody or ligand. The choice of partitioning method will depend on properties of the target and the nucleic acid and can be made according to principles and properties known to those of ordinary skill in the art.

-14-

"Amplifying" means any process or combination of process steps that increases the amount or number of copies of a molecule or class of molecules. In preferred embodiments, amplification occurs after members of the test mixture have been partitioned, and it is the facilitating nucleic acid associated with a desirable product that is amplified. For example, amplifying RNA molecules can be carried out by a sequence of three reactions: making cDNA copies of selected RNAs, using the polymerase chain reaction to increase the copy number of each cDNA, and transcribing the cDNA copies to obtain RNA molecules having the same sequences as the selected RNAs. Any reaction or combination of reactions known in the art can be used as appropriate, including direct DNA replication, direct RNA amplification and the like, as will be recognized by those skilled in the art. The amplification method should result in the proportions of the amplified mixture being essentially representative of the proportions of different sequences in the mixture prior to amplification. It is known that many modifications to nucleic acids are compatible with enzymatic amplification. Modifications that are not compatible with amplification can be made after each round of amplification, if necessary.

"Randomized" is a term used to describe a segment of a nucleic acid having, in principle, any possible sequence over a given length. Randomized sequences will be of various lengths, as desired, ranging from about eight to more than one hundred nucleotides. The chemical or enzymatic reactions by which random sequence segments are made may not yield mathematically random sequences due to unknown biases or nucleotide preferences that may exist. The term "randomized" is used instead of "random" to reflect the possibility of such deviations from non-ideality. In the techniques presently known, for example sequential chemical synthesis, large deviations are not known to occur. For short segments of 20 nucleotides or less, any minor bias that might exist would have negligible consequences. The longer the sequences of a single synthesis, the greater the effect of any bias.

A bias may be deliberately introduced into a randomized sequence, for example, by altering the molar ratios of precursor nucleoside (or deoxynucleoside) triphosphates in the synthesis reaction or the ratio of phosphoramidites in the chemical

-15-

synthesis. A deliberate bias may be desired, for example, to affect secondary structure, to introduce bias toward molecules known to have facilitating activity, to introduce certain structural characteristics, or based on preliminary results.

5 In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are
10 selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding
15 a base at any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs
20 between the target and those nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to the highest affinity nucleic acids exist in the candidate mixture, it is
25 generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate
30 mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

-16-

5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate an enriched candidate mixture. The SELEX Patent Applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein.

SELEX provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research. The present invention applies the SELEX procedure to more complicated tissue targets.

Negative selection (Counter-SELEX) is optionally employed before, during or after the Tissue SELEX process. The negative selection provides the ability to discriminate between closely related but different tissue types. For example, negative selection can be introduced to identify nucleic acid ligands that have a high specificity for a tumor cell but do not recognize the cognate normal tissue. Similarly, nucleic acid ligands can be identified which specifically recognize atherosclerotic arterial tissue but not normal arterial tissue. Nucleic acid ligands which recognize fibrin, but not fibrinogen can also be identified by this method. Additionally, nucleic acid ligands to a cell type which express a certain receptor can be counter-selected with a cell line engineered not to express the receptor (or other such macromolecule).

One of ordinary skill in the art will readily understand that various mechanisms can be employed to accomplish this negative selection. The following examples are provided mostly for illustrative purposes and are not meant in any way as limiting the procedures of negative selection. Negative selection or Counter-SELEX

-17-

methods were first described in United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands that Discriminate Between Theophylline and Caffeine", which is herein incorporated by reference. A particular implementation of negative selection is embodied using

5 equilibrium partitioning. In this method, two cell lines or other tissue types are separated by a semi-permeable membrane (0.45- 0.90 μ m pore size) in an equilibrium dialysis chamber; one cell line is the neoplastic target cell line, the other, the normal tissue used for the negative selection. The choice of cell or tissue type for the negative selection will be determined by the specific end results desired and will sometimes

10 consist of a non-malignant cell line of the same tissue type as the neoplastic target. For other experiments, various normal cell types could be combined to create the negative epitope "sink." The random pool of nucleic acids is placed into the dialysis chamber (on the side of the normal cells; this avoids background from high avidity targets which are common to both the tumor and normal cells) and allowed to

15 equilibrate between the two cell lines. Those nucleic acid sequences that remain bound to the target cell line or tissue at equilibrium are selectively recovered and amplified for the next round of SELEX.

This example of negative selection methodology is quite powerful. First, equilibrium dialysis negative selection allows the positive and negative selection

20 to be carried out *simultaneously*. Second, the stringency of the negative selection can be varied through the alteration of the relative amounts of "positive" and "negative" cells placed on each side of the dialysis membrane. These two characteristics of equilibrium dialysis negative selection allow precise control over the evolution of nucleic acid ligands specific for the target cell or tissue type.

25 This same type of equilibrium partitioning negative selection can be carried out with adherent cell lines. In this embodiment, monolayers of target and negative cells or tissues are plated in different wells of a multi-welled plate. After adherence, media, along with an oligonucleotide pool, is added such that the wells are connected by the volume of cell media. After equilibration of the oligonucleotide

30 pool, those sequences bound by the target cell line or tissue type would be isolated and amplified for the next round of SELEX.

-18-

The equilibrium negative selection strategies above offer a powerful way of generating nucleic acid ligands to tissue targets and especially tumor associated antigens (TAAs).

5 Additionally, there are several other negative selection methods, which could be classified as "post-SELEX screening procedures." The most simple of these procedures is the testing of individual nucleic acid ligands (those sequences generated by tissue SELEX and demonstrated to be high-affinity ligands for the tissue target) against normal tissue for cross-reactivity. However, this approach is a tedious and time-consuming process.

10 A more fruitful "post-SELEX" method is to perform a negative selection, for example using a normal tissue as the negative selection target, on a pool that has already been evolved from a SELEX against a desirable complex tissue target, for example a transformed cell line. This example would suggest the performance of two to three negative selections on a normal tissue using a late-round, highly evolved
15 pool from a SELEX of a transformed cell line. The binding of certain sequences to the normal tissue would be used to subtract these sequences from the evolved pool. This method allows one to quickly eliminate from several hundred to several thousand nucleic acid sequences that show a high affinity for those targets common to both the normal and the transformed cell lines.

20 Another "post-SELEX" screening method is a variation of the photocrosslinking experiment described in Example two below. As an example, it is possible to synthetically incorporate a highly photoreactive nitrene group (which is also iodlatable) on the 5' end of a PCR primer used in the tissue SELEX protocols. Late-round pools from for example, a tumor cell line SELEX would be amplified with
25 this photoactivatable (and ^{125}I -labeled) primer, and this sequence pool would then be irradiated in the presence of the tumor cell line, and in the presence of normal tissue. Membrane proteins would be isolated and solubilized for analysis on an SDS gel. One would expect to see many different protein epitopes tagged by specific oligonucleotide sequences, for both the tumor and the normal cell lines. A few tagged targets will be
30 unique to the tumor cell line. Because the oligonucleotides have been photochemically linked to the protein targets in a manner which does not destroy the base sequence of

-19-

the oligonucleotide, it is possible to isolate a tumor-specific band from an SDS gel, and use PCR to recover a specific sequence motif that recognizes a particular tumor antigen. Thus, in one step, it will be possible to remove from a pool oligonucleotide sequences that recognize possibly hundreds of cell surface antigens, leaving one or a few families of sequences that bind specifically to a single tumor-specific antigen.

As described above, the Tissue SELEX methods can include the identification of macromolecules which comprise new epitopes on the tissue target. The nucleic acid ligand to the new epitope component of the macromolecule can be employed to purify, identify and characterize the macromolecule. The new macromolecule can be a previously unknown protein or peptide, lipid, carbohydrate, etc. Virtually any molecule that is part of the molecular make-up of a tissue can be identified by the Tissue SELEX process.

In order to fully exploit this aspect of the invention, it is important to develop strategies for the purification and identification of new macromolecules which comprise the new epitopes and to determine the roles these new macromolecular components of the tissue play in biological systems. The methods for purifying new macromolecules are well-known, especially in the art of protein purification. These standard purification methods include crosslinking, affinity chromatography, peptide microsequencing, Edman sequencing, mass spectrometry, and cDNA library searches.

The following discussion describes this process as it would be applied to the identification of a new tumor-associated antigen (TAA). For the purposes of this discussion, a TAA is a macromolecule that is expressed on a tumor cell, but not on a similar normal cell. A TAA may or may not be immunogenic. A TAA is merely one example of the kinds of macromolecules which can be identified by the Tissue SELEX process and simply used for illustrative purposes. However, it is readily apparent that this process can be extrapolated to any new macromolecule identified by the Tissue SELEX process.

As applied to TAAs, the identification of new TAAs by the Tissue SELEX process is composed of two main parts: one, developing strategies for the purification and identification of new TAAs, and two, the elucidation of the role these

-20-

tumor antigens play in cancer (i.e., determining the biological significance of each particular TAA in the development and progression of a particular cancer).

The steps of purification and identification of most of the TAAs should be straightforward and understood by one skilled in the art of protein purification. As with antibodies, SELEX provides a reagent—a high-affinity ligand specific for the tumor antigen—that is incredibly useful for the purification of the antigen from whole cells or other tissues. As a non-limiting example, most antigens will be amenable to some type of photo-affinity crosslinking as described in the RBC ghost SELEX experiments of Example 1 or in the negative selection strategies section above.

Specific crosslinking of the TAA, using a photoactivatable oligonucleotide with a 3' biotin conjugate will allow one-pass purification of the TAA target using streptavidin coated beads. An alternative method to this purification strategy is to use a column-bound high-affinity nucleic acid ligand to affinity purify the TAA target from solubilized target cell membrane preparations.

There are many compelling reasons to believe that the method provided herein for identifying macromolecules that comprise new epitopes on tissues offers distinct advantages over traditional methods of new macromolecule discovery. Again, the following discussion will be directed to tumor-associated antigen discovery, but one will readily understand that it can be broadly extrapolated to all new macromolecule discovery.

As applied to tumor-associated antigens, one must fully consider that all that is known about tumor antigens has been derived from the immune system's reaction to particular antigens; science has depended on the particular restrictions of the immune system, and the system's repertoires to distinguish antigenic differences between neoplastic and normal tissue. It is entirely possible that other tumor antigens exist that are not subject to immune response. Some investigators have hypothesized that there may in fact be many antigenic differences between cancer and normal tissue, which are, unfortunately, not immunogenic.

The SELEX methodology provides an improved way to identify TAAs that avoids the restrictions posed by the immune system:

-21-

a. SELEX can actually provide a deeper search of TAAs than can the entire potential antibody repertoire of an organism— the size of the nucleic acid libraries used in SELEX is unrivaled by any biological system;

5 b. SELEX provides nucleic acid ligands to targets, including those which are not antigenic to the immune system because of tolerance. Many of the TAAs which have been identified are oncofetal— they are antigens expressed at some point during development or cell differentiation. As prior “self” antigens, they elicit no overt immune response because of earlier immune system tolerization. A SELEX-based search for TAAs avoids the circular nature of using the immune system
10 as a means of identifying tumor antigens;

c. SELEX nucleic acid ligands have been shown to be exquisitely sensitive to target conformation. While most antibodies recognize conformational, or discontinuous epitopes, antibody functional epitopes are composed of only a few amino acids. The potential binding surface of an oligonucleotide ligand is much larger
15 than that of an antibody variable region, and may provide greater conformational discrimination of large targets. Additionally, cross-reactivity for SELEX ligands is substantially less of a problem than for monoclonal antibodies. A considerable set of restrictions also controls T-cell mediated tumor responses. These immune system limitations provide important biological functions; however, they limit the immune
20 system’s power for TAA identification.

d. SELEX is possibly more sensitive to small quantities of antigen than the immune system. Although the immune system’s threshold for reactivity has been estimated to be 200 copies/cell for an antigenic MHC-presented peptide, a B-cell antibody response (necessary for any antigen that is not a peptide— carbohydrates,
25 lipids or conformational antigens) to a monovalent target requires antigen concentrations of about 100 mM. SELEX can generate ligands to TAA targets with a low representation on the cell surface;

e. SELEX provides a rapid and thorough method of TAA discovery. Screening of monoclonal antibodies to tissue sections, and purification and
30 identification of MHC peptides are painstaking processes that set practical limits on

-22-

the depth and completeness of searches for TAAs. Tissue SELEX experiments take a much abbreviated length of time.

Nucleic acid ligands to tissue targets or the tissue epitopes identified by the method of the invention are useful as diagnostic reagents and as pharmaceuticals.

- 5 The nucleic acid ligands are also useful for the identification of new macromolecules. The nucleic acid ligands are useful in any application that would be suitable for use of an antibody.

- As diagnostic reagents, the ligands or tissue epitopes can be used in both *in vitro* diagnostics and *in vivo* imaging applications. The SELEX method
10 generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic applications. SELEX identifies nucleic acid ligands that are able to bind targets with high affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek for a diagnostic ligand. Details regarding use of the ligands in
15 diagnostic applications is well known to one of ordinary skill in the art. Nucleic acid ligands that bind specifically to pathological tissues such as tumors may have a role in imaging pathological conditions such as human tumor imaging and even therapeutic delivery of cytotoxic compounds or immune enhancing substances.

- The nucleic acid ligands of the present invention may be routinely
20 adapted for diagnostic purposes according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt any
25 nucleic acid ligand by procedures known in the art to incorporate a labelling tag in order to track the presence of a ligand. Such a tag could be used in a number of diagnostic procedures.

- Specifically, oligonucleotide ligands with high specificity for particular tumor antigens could become as important as monoclonal antibodies for the detection,
30 imaging, and surveillance of cancer. Modified nucleic acid ligands show nuclease resistance in plasma, and the use of 5' and 3' capping structures will provide stability

-23-

in animals that rivals that of monoclonal antibodies (and without the immunogenicity of animal-derived MAbs). Radionuclides, magnetic compounds, and the like can be conjugated to tumor-specific oligonucleotides for cancer imaging. SELEX tumor ligands can also be used to determine if these tumor antigens are sloughed off tumors, and are detectable in the plasma like PSA.

The nucleic acid ligands to tissue targets or newly identified macromolecules components of tissue are also useful as pharmaceuticals. Therapeutic uses include the treatment or prevention of diseases or medical conditions in human patients. Therapeutic uses also include veterinary applications. The ligands can bind to receptors and be useful as receptor antagonists. Conversely, under certain circumstances the ligands can bind to receptors and cause receptor capping and act as receptor agonists.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

Standard formulations can be used for the nucleic acid ligands of the invention and are known to one of ordinary skill in the art.

The following examples provide a non-limiting description of the present invention. Example One describes obtaining ssDNA ligands to the complex tissue target red blood cell ghosts. The red blood cell ghost comprises a finite set of membrane-bound epitopes and is a non-living target which remained unchanged over the period of the selection. Ligands to RBC ghosts have numerous uses including, but not limited to, the ability to in vivo image extravascular blood as is desirable for head or retroperitoneal injuries or to extend the vascular half-life of other ligands that may be attached to the RBC ghost ligand. Example Two describes the identification of a macromolecule component on the RBC ghost using a ligand obtained in Example One. Example Three demonstrates that red blood cell ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of

-24-

the target cell membrane. Example Four describes the identification of and enrichment for high affinity nucleic acid ligands which bind individual components of a complex macromolecular target. Example Five describes obtaining ssDNA ligands to a glioblastoma cell line. High affinity and specificity nucleic acid ligands were isolated that may interact with tumor-associated (or tumor-specific) antigens, or mimic cytokines in their interactions with cell surface receptors causing cell morphology changes. Ligands to glioblastoma cell lines have numerous uses including, but limited to, in vivo imaging of glioblastomas, therapeutic localization of the ligand or other therapeutic agents that are attached thereto. Example Six describes ssDNA ligands to a human lymphoma cell line.

Example One

ssDNA Ligands to Red Blood Cell Ghosts

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target human red blood cell ghosts (RBC ghosts). Red blood cell ghosts are erythroid cells which have been lysed, purged of their cellular contents and preferentially resealed in a right-side-out manner (Steck et al. (1994) Biochemistry 10: 2617-2624). Red blood cell ghosts were the first complex tissue target on which in vitro selection was performed. The red blood cell ghost is one of the least complicated tissue targets and yet is still orders of magnitude more complex than the pure proteins or small molecules previously used for SELEX procedures. The red blood cell ghost comprises a finite set of membrane-bound epitopes and is a non-living target which remained unchanged over the period of the selection. Ligands to RBC ghosts have numerous uses including, but not limited to, the ability to in vivo image extravascular blood as is desirable for head or retroperitoneal injuries or to extend the vascular half-life of other ligands that may be attached to the RBC ghost ligand.

Briefly, the RBC ghost SELEX was carried out with single-stranded DNA for selection, using a 30-base randomized region. The single-stranded DNA pool was incubated with RBC ghosts, and the tighter-binding sequences were partitioned from the rest of the pool by filtering the reaction through nitrocellulose filters. 25 rounds of selection were carried out, using a decreasing concentration of

-25-

ghosts as the SELEX experiment progressed. The 25th round pool was cloned and sequenced according to standard procedures. Listed in Table 1 are the 66 sequences isolated from the 25th round pool (SEQ ID NOS: 5-70). Approximately 60% of these sequences can be classified into seven sequence-specific motifs, there is one class of
5 pyrimidine-rich sequences (12%), and the other 19% are "orphans," showing no similarity to other sequences.

Binding behavior of round 0 and round 25 pools, and selected clones shows that the round 25 pool binds significantly better than the starting pool, and several of the motif 1 clones bind better than the round 25 pool. All sequences tested
10 for binding so far show similar binding to whole red blood cells, so it is believed that the SELEX ligands have evolved to membrane targets on the extracellular side of the RBC ghosts.

A. MATERIALS AND METHODS

Red blood cell ghosts

15 Red blood cell ghosts are erythroid cells which have been lysed, purged of their cellular contents and preferentially resealed in a right-side-out manner (Steck et al. (1994) Biochemistry 10: 2617-2624). The concentration of protein in the preparation was measured with Coomassie brilliant blue G-250 (Bio-Rad).

Synthesis of initial pool of ssDNA

20 10 pmol of template with 30 random nucleotides flanked by fixed sequences complementary to the primers (SEQ ID NO: 1) was PCR amplified for 25 rounds in 10 mM Tris-HCl, pH 8.6, 50 mM KCl, 2.5 mM MgCl₂, 170 mg/ml BSA, 1 mM dNTPs, 0.5 units/ml *Taq* DNA polymerase and 5 mM each primer (5'-GGGAGCTCAGAATAAACGCTCAA-3' (SEQ ID NO: 2) and
25 5'-BBBGATCCGGGCCTCATGTCTGAA-3' (SEQ ID NO: 3), where B=biotin). A similar reaction contained 1 pmol of template, 0.1 mM dCTP and 1.25 mM [α -³²P]dCTP (800 Ci/mmol) to produce internally labeled ssDNA for monitoring the binding affinity of the pool. Non-biotinylated, ssDNA was purified from the larger biotinylated strand by electrophoresis in 8% polyacrylamide gels containing urea.

The SELEX Protocol

30

-26-

40 pmol unlabeled ssDNA and a trace amount of radioactively labeled ssDNA were denatured by heating at 70°C for 5 min in 200 µl PBS (pH 7.3) and renatured at 0°C for 10 min. Pre-filtration of the DNA solution was used to counter-select sequences that might bind to nitrocellulose. After washing the filter with 300 µl PBS, the ssDNA molecules passed through the filter were divided into 50 µl aliquots. An equal volume of PBS containing various concentrations of RBC ghosts (0-1.72 mg/ml total protein) was added to each aliquot. The mixture was incubated for 20 min at room temperature then filtered through nitrocellulose. The filters were washed with 5 ml PBS and the amount of radioactively labeled ssDNA retained was measured by scintillation counting. The ssDNA was isolated from the filter that retained 5-10 times the radioactivity bound to the background control filter and was amplified by PCR for the next round of selection.

Nitrocellulose filter binding assays

The nitrocellulose filter partitioning method was used as described in SELEX Patent Applications to determine the affinity of nucleic acid ligands for RBC ghosts and for other proteins. Filter discs (nitrocellulose/cellulose acetate mixed matrix, 0.45 µm pore size, Millipore) were placed on a vacuum manifold and washed with 5 ml of TBSC buffer under vacuum. Reaction mixtures, containing ³²P labeled nucleic acid pools and RBC ghosts, were incubated in TBSC for 5 min at 37 ° C, filtered, and then immediately washed with 5 ml TBSC. The filters were air-dried and counted in a Beckman liquid scintillation counter without fluor. Dissociation constants for single RBC ghost ligands were determined by Scatchard analysis (Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51:660-627; Robb, R.J., Munck, A., and Smith, K.A. (1985) J. Immunol. Methods 81:15-30), using constant ghost concentrations and varying the concentration of nucleic acid ligand. Scatchard analysis was performed using nitrocellulose partitioning of bound ligand from unbound ligand. For comparisons between random and evolved nucleic acid ligand pools, and for ligand/ligand comparisons, standard filter binding assays were used as described in the SELEX patent applications.

Cloning and nucleotide sequence determination

-27-

Individual DNA molecules were isolated from the round 25 pool by PCR amplification with primers that introduce *Bam*HI and *Hind*III restriction sites at the 5' and 3' ends of the DNA. Restriction digested PCR products were ligated into pUC18 and introduced into *E. coli* strain SURE (Stratagene) by electroporation.

- 5 Plasmids were isolated and the nucleotide sequences in the inserted DNAs were determined by standard dideoxynucleotide methods. The sequences were searched for patterns in their primary sequences and in their possible secondary sequences both by inspection and with the aid of computer algorithms.

10

B. RESULTS OF THE SELEX PROCEDURE

Clones

- As described in Section A, ssDNA with 30 randomized positions was
15 used in SELEX with RBC ghosts as the target. The affinity of the ssDNA population for the membranes increased over twenty-five rounds of selection and amplification. The round 25 PCR products were cloned and the nucleotide sequences of 66 individuals were determined as shown in Table 1 (SEQ ID NO: 5-70). Eight clones contained one 8 and one 11 nucleotide consensus sequence separated by 3 to 14 bases
20 (SEQ ID NOs: 5-12). This group of sequences has been termed motif I sequences. Several of these clones are likely to have arisen from a single progenitor sequence by PCR mutagenesis (ie, 20, 121 and 117). One of the clones (clone 25)(SEQ ID NO: 12) in this group may use a portion of the 5'-end fixed region to complete the consensus sequence. A region of this fixed sequence and the consensus sequence differ by only
25 two nucleotides. Binding analysis of portions of the motif I sequences have defined the minimum binding sequence as the region containing no more than the 8-base and 11-base consensus sequences. Two synthetic truncate sequences have been made from the motif I sequences c56t (SEQ ID NO: 4) (from parent 56) and c20t (SEQ ID NO: 236) (from parent 20). The extremely high similarity between all the motif I sequences
30 has prevented a phylogenetic analysis of the sequences and data on the secondary structure of this motif has not been obtained as shown in Figure 3.

-28-

Another group of 7 sequences (SEQ ID NOS: 22-25 and 35-37) contain an 18-base conserved primary sequence and share additional secondary structural elements. Computer folding algorithms and phylogenetic analysis predict a hairpin-bulge-stem structure for these sequences as shown in Figure 3. These sequences have been termed the motif II sequences. Two synthetic truncated ligands have been made for this sequence motif, c16t (SEQ ID NO: 237) (parent 16) and c79t (SEQ ID NO: 238) (parent 79).

An additional group of 10 sequences share a common region of 13 bases, surrounded by additional conserved secondary structural elements. Computer folding algorithms and phylogenetic analysis predict a stem-bulge-stem structure for this group of sequences, called the motif III sequences (SEQ ID NOS: 18-21; 28-30; 40-42) as shown in Figure 3. The similarity between the members of the motif III sequences becomes even more substantial at the secondary structure level, for the motif III ligands accomplish this structure in two different *circularly permuted* ways. Figure 3 illustrates this permutation for two motif III truncate ligands, c53t (SEQ ID NO: 240) (parent 53) and c111t (SEQ ID NO: 239) (parent 111).

Three more sequence motifs have been defined by sequence homology. Motif IV contains 5 members, motif V has 5 members, and motif VI 2 members as shown in Table 1. The possible secondary structures for these sets of ligands has not yet been determined.

Twenty of the sequences show no large sequence homology to other sequences and are termed orphans. While several identical clones lie within this group, these clones most likely arose from a single progenitor sequence and do not represent another "motif."

The final group of sequences showed extremely high pyrimidine content (77-90 %), and no common secondary structure has been proposed.

Affinities

The binding behavior of round 0 and round 25 pools, and a selected number of round 25 clones have been tested. The round 25 pool binds approximately 10-fold better than the starting pool, and several of the motif I clones bind 100-fold

-29-

better than the round 0 pool. All sequences tested for binding show similar binding to whole red blood cells, and therefore the inventors hereof believe that ligands have been selected to membrane targets on the extracellular side of the RBC ghosts.

A synthetic twenty-two nucleotide truncate of clone 56 (c56t)(SEQ ID NO: 4) that contains only the consensus sequences with four intervening nucleotides retained most of the binding affinity exhibited by the entire ssDNA sequence. A Scatchard plot analysis of c56t measured 1600 binding sites per cell, and a calculated dissociation constant of 4 nM for the target presented on the RBC ghosts. Truncate ligands from motifs II and III have not yet been analyzed for binding to the ghosts, but the photoaffinity studies of these ligands shown in Examples 3 and 4 indicates that their dissociation constants are as good or better than c56t. The pyrimidine-rich clones had affinities that were higher than the round 25 pool but lower than the consensus clones.

Example Two

Identification of Macromolecule Component on RBC Ghost

In order to confirm that the c56t ligand (SEQ ID NO: 4) recognizes a single, distinct target on RBC ghosts, a series of short-wavelength UV crosslinking experiments were done in an effort to photochemically link the c56t ligand to its membrane target through thymidine crosslinking. As controls, two 22-base DNA oligonucleotides of the same base composition, but scrambled in sequence were also crosslinked to the RBC ghost target. Briefly, the target recognized by c56t was identified by short wavelength (254 nm) UV crosslinking experiments. 5' ³²P end labelled truncate ligand c56t, and two control oligonucleotides of the same length and base composition (but with the primary sequences scrambled using a "shuffling" computer algorithm), were irradiated in the presence of RBC ghosts. The ghost membrane proteins were fractionated using denaturing SDS gel electrophoresis, and the presense of crosslinked ligand detected by autoradiography of the dried gel. The results are shown in Figure 1. Autoradiography indicated a single specific crosslinked product for c56t (all three oligos show slight crosslinking to two other RBC ghost proteins). The c56t ligand, but not the two controls, selectively labels an RBC ghost

-30-

membrane protein with an apparent molecular weight of 105 kDa. Silver staining of this protein target indicates that it is not an abundant protein.

A similar short wavelength photoaffinity crosslinking experiment was performed using both specific and non-specific nucleic acid competitor in the photocrosslinking

5 reaction (Figure 3). The addition of a 10^3 molar excess cold c56t in the reaction abolished crosslinking to the 105 kDa ghost component. However, the addition of a 10^3 molar excess of cold motif II sequence c16t did not affect the crosslinking of c56t. This "cross competition" experiment demonstrates the incredible affinity and specificity of the truncate ligand c56t with its protein target.

10 Additionally, the product of the photoaffinity crosslinking reaction was examined under both reducing and non-reducing SDS-PAGE as shown in Figure 2. Under reducing conditions, the crosslinked protein runs with an apparent molecular weight of 105 kDa. Under non-reducing conditions, the crosslinked protein migrates at about 210 kDa, and suggests that the crosslinked protein is present on the ghost
15 membrane as a disulfide-linked hetero- or homo-dimer. At present, only two human CD antigens that are disulfide bonded homodimers with monomer molecular weights within the range of 90-110 kDa are known, and only one is present on red blood cells and its direct progenitors. This antigen is the transferrin receptor (with a monomer molecular weight of 95 kDa). A definitive demonstration of the identity of the protein
20 crosslinked by c56t is under investigation.

Example Three

Red Blood Cell Ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of the target cell membrane

25 A key assumption of tissue SELEX is that nucleic acid selection of collections of large macromolecular structures should result in the generation of high affinity ligands to all independent binding sites on these structures. Since cells or tissues are many magnitudes of order larger than a purified protein target, the number of these independent binding sites should be large. In brief, this theory predicts that
30 selection of multiple targets produces ligands with multiple binding specificities.

-31-

Thus the selection of red blood cell ghosts should result in the evolution of high affinity nucleic acid ligands to more than one, and potentially all protein targets present on the membrane surface. In an effort to provide definitive proof of this hypothesis, truncate ligands from the first two red blood cell ghost sequence classes (motif I and II; see Figure 3) were affinity photocrosslinked to the ghost membranes. Truncates c56t (motif I) (SEQ ID NO: 4) and c16t (motif II) (SEQ ID NO: 237) were made synthetically, with the addition of a primary aliphatic amine (with a six carbon spacer group) on the 5' end of each molecule. This amino group was used to conjugate the truncate ligands to the phenyl azide photoreactive molecule sulfo-HSAB (N-hydroxysulfo-succinimidyl 4-azidobenzoate, Pierce Chemical Company). Additionally, these molecules were radiolabeled on their 3' end using alpha 32^p ddATP. The truncate ligand conjugates were mixed with ghosts and photocrosslinking carried out using a 308 nm excimer laser as shown in Figure 4. To demonstrate high affinity and specificity, the photoreactive truncates were irradiated with the ghosts in the presence of cognate or non-cognate unirradiated, unconjugated truncate.

The motif I truncate ligand c56t specifically labels a dimer protein band of apparent molecular weight of 105 kDa, the identical protein band labeled by this truncate using short wavelength UV photocrosslinking. This photoaffinity crosslinking can be prevented by the addition of 10⁴ molar excess of "cold" c56t, but not by the addition of 10⁴ molar excess of cold c16t. Similarly, the motif II truncate specifically labels a protein of apparent molecular weight of 40 kDa. This crosslink can be prevented by the addition of cold c16t but not by cold c56t. Thus, it is clear the red blood cell ghost SELEX has produced high affinity and high specificity ligands to more than one macromolecular component of the target cell membrane.

This photoaffinity analysis has now been carried out for all truncate ligands shown in Figure 3. The motif I truncate c20t (SEQ ID NO: 236) specifically labels the same protein dimer band as the motif I truncate c56t, and the motif II truncate c79t (SEQ ID NO: 238) labels the same 40 kDa protein band as the motif II truncate c16t. The two motif III truncate ligands c53t (SEQ ID NO: 240) and c111t (SEQ ID NO: 239) specifically label a group of three proteins ranging in molecular

-32-

weight from 42-55 kDa, and presumably these proteins are physically associated as a protein complex on the ghost membranes. This consistent pattern of identical photoaffinity crosslinking behavior *within* sequence motifs, and different protein bands crosslinking *among* motifs is very strong proof of the fundamental hypothesis of tissue

5 SELEX— multiple targets result in ligands with multiple specificities.

Example Four

Identification of and enrichment for high affinity nucleic acid ligands which bind individual components of a complex macromolecular target

10 After the generation by tissue SELEX of high affinity ligands to many targets within a complex mixture, it is desirable to be able to screen this large pool of sequences for those nucleic acid molecules which recognize a particular, discrete target within the complex mixture. A method for this procedure has been developed for the red blood cell ghost SELEX which has been termed "pool deconvolution." The pool

15 of sequences from the final round of the RBC ghost SELEX (round 25) was amplified using internal radiolabel and a "sense strand" PCR primer which carried the same primary amine, six carbon spacer described in Example Three at its 5' end. Thus, every sequence in the purified ssDNA pool contained this primary amino group at their 5' end. The pool of sequences was conjugated to the phenyl azide compound

20 sulfo-HSAB, purified, and incubated with the RBC ghosts in the presence of 10^3 molar excess of non-specific nucleic acid competitor. The mixture was irradiated using a 308 nm excimer laser and the crosslinked products separated by SDS-PAGE.

The crosslinking pattern of the final round pool is shown in Figure 5. One can clearly see that many different proteins present in the ghost membrane have

25 been specifically photolabeled by the pool sequences. The SDS-PAGE separated products were electroblotted to a nitrocellulose filter, and sections of the filter which corresponded to four different crosslinked proteins were excised and placed in PCR reactions for amplification of the pool sequences which crosslinked to the particular protein selected. This "deconvolution SELEX" was carried out for three rounds, and

30 the results of the selections are shown in Figure 5. Lanes numbered 5, 6, 7, and 8 correspond to the four selected protein bands as indicated on the round 25 lane. The

-33-

three rounds of selection has produced excellent enhancement for sequences which can specifically photocrosslink to selected ghost membrane proteins. The pools used to produce lanes 5 and 8 are both close to becoming completely specific for the selected proteins. The stringency of further selections will be increased by using high concentrations of non-specific competitor nucleic acid and by competing a particular pool (such as that for band 5) with cold, non-conjugated fractions of the remaining three pools. Such a scheme should allow the selective removal of sequences that are common to two or more pools. For example, competing the photocrosslinking of the pool for band 5 with cold material from the band 6, 7, and 8 pools should eliminate the common crosslinking between the band 5 pool and the other pools. When the selection is completed, the isolated DNA for a particular protein band can be readily sequenced by standard methods, allowing one to correlate particular nucleic acid sequences with high affinity binding to a discrete protein. This deconvolution technique is a powerful method for screening high affinity tissue SELEX pools for sequences which bind a particular target of interest.

Example Five

ssDNA Ligands to Glioblastoma U251 Cell Line

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target glioblastoma cell line U251, which is derived from human brain tumor (Hum. Hered. (1971) 21:238). High affinity and specificity nucleic acid ligands were isolated that may interact with tumor-associated (or tumor-specific) antigens, or mimic cytokines in their interactions with cell surface receptors causing cell morphology changes. Many of the protocols used in this example are outlined in Example One or are slightly varied as described below. Ligands to glioblastoma cell lines have numerous uses including, but not limited to, in vivo imaging of glioblastomas, therapeutic localization of the ligand or other therapeutic agents that are attached thereto.

In this tissue SELEX example, a fluorescent-labeled single-stranded DNA library with 34 nucleotide randomized region was used (SEQ ID NO: 71). The fluorescent-labeled ssDNA was purified by denaturing polyacrylamide gel. The sequences of primers and template are as follows:

-34-

5'-primer: 5'-F-GCCTGTTGTGAGCCTCCT-3' (F: fluorescein) (SEQ
ID NO: 72)

3'-primer: 5'-GGGAGACAAGAATAAGCG-3' (SEQ ID NO: 73)

template:

55'-GCCTGTTGTGAGCCTCCT-N34-CGCTTATTCTTGTCTCCC-3' (SEQ ID NO:
71)

Briefly, the SELEX procedure was as follows. One to 10 million glioblastoma cell line U251 cells were washed twice in a culture flask with 20 mL cold RPMI-1640 serum-free medium at 4°C. 50-100 picomoles of ssDNA in 100 μ L PBS buffer was heated at 90°C for 5 minutes and put on ice for 5 minutes. The ssDNA pool was added to the cell culture in 20 mL RPMI-1640 medium along with 20-40 fold excess sonicated sperm DNA and yeast tRNA (molar ratio 1:1). The solution was incubated at 4°C for 20 minutes with gentle shaking. The cells were washed twice with 20 mL cold RPMI-1640 medium to remove the free oligonucleotides. The cells were trypsinized with 1 mL of 0.25% trypsin. The solution that contains cells and oligonucleotides was collected to a 2 mL tube, boiling at 95°C for 5 minutes, followed by phenol extraction and ethanol precipitation. The recovered ssDNA was used for PCR amplification. Through 20 rounds of selection, the binding affinity of the final pool was significantly increased comparing with that of the starting material. The affinity increase was revealed by Scatchard graph. The round-20 pool was cloned into pUC18 vector by DUG cloning as described by Rashtchain *et al.* (Anal. Biochem. (1992) 206:91). About 158 sequences were obtained, which can be grouped into 22 subfamilies and are shown in Table 2 (SEQ ID NOs: 74-232).

Example Six

ssDNA Ligands to Human Lymphoma Cell Line

This example demonstrates the ability to obtain ssDNA ligands to the complex tissue target human lymphoma cell line CEMss, which is a CD4 positive cell line (Foley et al., Cancer (1965) 18:522). Many of the protocols used

-35-

in this example are outlined in Example One or are slightly varied as described below.

In this tissue SELEX example, fluorescein labeled single-stranded DNA molecules were used for the generating of combinatorial library. The fluorescein-labeling allows for image of oligonucleotides binding to the cell surface and for the purpose of flow cytometry. The sequences of primers and templates are as follows:

5'-primer: 5'-F*-GCCTGTTGTGAGCCTCCT-3' (F*=fluorescein)
(SEQ ID NO: 233)

3'-primer: 5'-GGGAGACAAGAATAAGCG-3' (SEQ ID NO: 234)
template:

5'-GCCTGTTGTGAGCCTCCT---N₃₄---CGCTTATTCTTGTCTCCC-3'
(SEQ ID NO: 235)

Briefly, the SELEX procedure was as follows. The target cell line was the human lymphoma cell line CEMss, which is CD4 positive. 5X10⁶ cells were washed twice with 10 mL of cold PBS buffer in a 15 mL conical tube. The cells were resuspended with 1 mL PBS and stored on ice. 50-100 picomoles of fluorescein-labeled (and ³²P-internally-labeled by PCR) single-stranded DNA (SEQ ID NO: 235) in 100 μL PBS was heat denatured at 90°C for 5 minutes, and was kept on ice for 5 minutes. Incubate the single-stranded DNA together with 20-50 fold excess competitor yeast tRNA and sonicated denatured sperm DNA (ratio: 1 to 1), with cells at room temperature for 20 minutes with gentle shaking. Load the reaction solution on top of 0.5 mL of binding oil (84% silicon oil and 16% paraffin oil), spin at top speed for 15 seconds, immediately freeze in dry ice/ethanol. Cut the bottom tip of the tube off and put the tip in a 2 mL tube, add 100 μL water, 100 μL 7 M urea, and 400 μL phenol, shake and boil for 5 minutes. Count the cpm, then shake for another 20 minutes, spin at top speed for 10 minutes, transfer the top phase to a new tube and ethanol precipitate. The recovered DNA was PCR amplified and purified on a denaturing gel. The fluorescein-labeled strand migrates slower. The recovered ssDNA was used for next round of SELEX.

-36-

The improvement of binding affinity was determined by binding assay. The reaction condition was as described above, with the exception that the reaction volume is 100 μ L, without the addition of competitor. After 12 rounds of selection the binding affinity increased compared to the zero round pool. The complexity of the round 12 pool is still relatively high and rounds will continue until the resulting complexity of the pool has somewhat decreased.

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Table 1
RBC Ghost SELEX

SEQ ID NO:	Starting Pool
1	gggagctcagaataaaacgctcaa[30N]ttcgacatgagggcccggtc
5	gggagctcagaataaaacgctcaaCTCAGTGGT----AGGTAACGGTTCAAGACGGGAttcgacatgagggcccggtc
6	gggagctcagaataaaacgctcaaCTCAGTGGT----AGGTAACGGTTATATCCGGAAttcgacatgagggcccggtc
7	gggagctcagaataaaacgctcaaAACTCAGTATA----AGGTAACGGTTCCAAACCCAGAttcgacatgagggcccggtc
8	gggagctcagaataaaacgctcaaACTCAGTAATGCCAAGGTAACGGTTCCCTTTtcgacatgagggcccggtc
9	gggagctcagaataaaacgctcaaACTCAGTAATGCTP-AGGTAACGGTTCCCTTTtcgacatgagggcccggtc
10	gggagctcagaataaaacgctcaaACTCAGTAATGCACCCAGTAACGGTTACATCTtcgacatgagggcccggtc
11	gggagctcagaataaaacgctcaaACTCAGTAGCA--AGGTAACGGTTTCAGATCCACTtcgacatgagggcccggtc
12	agctcagaataaaacgctcaa- GTCATTAACGGTTAGCCAGAGGACCGTGCCTtcgacatgagggcccggtc
4	aaCTCAGTGGT----AGGTAACGGTT
236	aaCTCAGTAATGCCCAAGGTAACGGTT
35	gggagctcagaataaaacgctcaaACGAATCGCATTGCCCAA-CGTTCGCC-AAGAttcgacatgagggcccggtc
36	gggagctcagaataaaacgctcaaCCGAATCGCATTGCCCAA-CGTTCGCC-AAGAttcgacatgagggcccggtc
37	gggagctcagaataaaacgctcaaTGTCGGATAAGTCGCCCAA-CGTTCGCC--ATTtcgacatgagggcccggtc
22	gggagctcagaataaaacgctcaaTAG-TTCGCCA-CGGTTGTCC-AATTGATCGTAttcgacatgagggcccggtc
23	gggagctcagaataaaacgctcaaTGG-TTCGCCAT-CGTTCGTCC-AATTGATCGTAttcgacatgagggcccggtc
24	gggagctcagaataaaacgctcaaT-G-TTCGCCATTCGTTCGTCC-AAGTGAACGTAttcgacatgagggcccggtc
25	gggagctcagaataaaacgctcaaTGAATTGCCCAA-CGTTCGCCGAA-TGATGCGTtcgacatgagggcccggtc
237	CGAATCGCATTGCCCAA-CGTTCGCC-AAGAttcg
238	cgctcaaTAG-TTCGCCA-CGGTTGTTC-AATTGAGCG

-37-

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pyrimidine-rich motif

-40-

TABLE 2
Glioblastoma Ligand Sequences

Sequences: (fixed regions not shown)

	Ligand NO:	Random Region	
5	GBI.1	GGCTGCTGAGTCCAGGGGCGATAACGGGCTTTG	74
	GBI.2	GGCTGCTGAGTCCAGGGGCGATAACGGGCTTTG	75
	GBI.120	GGCTGCTGAGTCCAGGGGCGATAACGAGCTTTC	76
	GBI.140	GGCTGCTGAGGCCAGGGGCGATAACCGCACTTT	77
	GBI.162	GGCTGCTGAGTCCAGGGGCGATAACGGCCTTTC	78
	GBI.4	TAGC GAACACAGGGGNCACAACTGGCTATCTCT	79
	GBI.8	TAGCAGAACACAGGGGNCACAACTGGCTATCTC	80
	GBI.33	TAGGCGAACACAGGGGTCCACAACTGGCTATCCC	81
10	GBI.124	TAGC GAACACAGGG TCAACAGCTCACACGGCC	82
	GBI.125	TAGC GAACGARCAGGTGCCCTGCTCTCAACTGGTTT	83
	GBI.99	TAGGCCGGAGGGACTAATAGCTTACAGCGCACTA	84
	GBI.76	TAGGCCGGAGGGACTAATAGCTTACAAGGCACTA	85
	GBI.42	TAGGAGCGCGAACAAACGGGGAGGTCTCACACTG	86
	GBI.23	TAGGGGNGNATAACAACAGGTCTGGTCAACAATG	87
	GBI.75	TAGGGCGGAGNGNGCGGTCTCTCTGGNNACACTC	88
	GBI.27	AGGCAGAAGTGAGCTTGGGCTCGCAACTCTCTCC	89
	GBI.29	AGGCNGTAG GNGCTAGGGNGNACTCGTATTCCTC	90
15	GBI.101	AGGCAGCAGTGA CTTGGA CGACAACAGCTATGTC	91
	GBI.156	AGGCAGTAGTGA CTTGGGCGCAGAGGAGGGTAGT	92
	GBI.189	AGGGCGCAGGG TCTAGGGCANCCAAACAGCTATTG	93
	GBI.145	AGGCGAAGGGN CTAGGGTGNACAGCAGCGGTGG	94
	GBI.10	NNNAGAGGGAAGACTTTAGGTTTCGGTTCACGTCC	95
	GBI.36	NNNAGAGGGAAGAC TTAGGTTTCGGTTCACGTCC	96
	GBI.41	CCCAGAGGGAAGACTTTAGGTTTCGGTTCACGTCCC	97
	GBI.73	NCCAGAGGGNAGACTTTAGGTTTCGGTTCACGTCC	98
	GBI.132	NNNAGAGGGAAGGCTTTAGGTTTCGGTTCACGTCC	99
20	GBI.170	NNNAGAGGGAAGACTTTAGGTTTCGGTTCACGTTC	100
	GBI.181	NNNAGAGGGNAGACTTTAGGTTTCGGTTCACGTCC	101
	GBI.14	GTGTGCAACAGAGCAGNNNTTGTCTAACATCACTT	102
	GBI.13	GGGGCGAACAGCAGCTACTCACAACATGTCCGGC	103
	GBI.26	GTGGCGAACACGGGTCAAGGGCTTCACAAATCTG	104
	GBI.35	ATGGCGAACACAGCAACTCGCTCACAACCTCTCTCC	105
	GBI.38	GTAGGCGAACACAGGTTGAGGCTTACACAGGGNT	106
	GBI.43	AGCGAACAACTGACTGACGGCAGGGTCAACACNNC	107
	GBI.52	TACGAACAACAGCATTACACAGGCCTTTTGTGTT	108
25	GBI.183	AGCGAGCAACATCTTTTCGCAACAGGTTTGGTTCC	109
	GBI.62	TTGGCGAACACAGCAACTCGCTCACAACATATCTT	110
	GBI.6	AGGTTGGGTAGGTTGG TGGAGGCGAACGTACCAA	111
	GBI.58	AGGTTGGGTAGGTTGG TGGAGGCGAACGTCTCTAA	112
	GBI.182	AGGTTGGGTAGGCTGG TGGAGGCGNACGTCCCAT	113
	GBI.141	AGGTTTCGC AGGCTGGCTGGAGGCGCGACCCAA	114
	GBI.37	GTTTTGACCG TAACAA TTGTTAAA GCTCCGGGNN	115
	GBI.61	GGTCTGATCG TAACAA TTGTTAAA GCTCCGGGNC	116
30	GBI.86	GGTTTGATCTCTAACAA TTGTTAAA GCTCCAGGC	117
	GBI.94	GGTCTGATCGCTAACAA TTGTTAAA GCTCCGGGGC	118
	GBI.104	GGTCTGATCG TAACAAATTGTTAAAAGCTCCGGGGC	119
	GBI.119	GTTTTG TCG TAACAA TTGTTAAA GCTCCGGGAC	120

-41-

	GBI.171	GGTCTGATCG TAACAG TTGTTAAAAGCTCCGGGCG	121
	GBI.187	GGTCTGATCG TAACAA TTGTTAA GCTCCGGGCG	122
	GBI.18	CCGCCAAGGGAGCTCTCCGAGCTCGGCGCCACTC	123
	GBI.60	NCNNCNAAGGAAGATCTCCGAGTTCGGCGTCACTG	124
	GBI.68	CTGCCGGGGAAGATCTCCGAGTTCGGCGTCACTG	125
	GBI.69	CCGCCAAGGAAGATCTCCGAGTTCGGCGTCACTG	126
	GBI.89	CNGCNAAGGAAGATCTCCGAGTTCGGCGTCACTG	127
5	GBI.123	CNGCCAAGGAAGATCTCCGAGTTCGGCGTCACTA	128
	GBI.185	CNNCNAAGGAAGATCTCC AGTTCGGCGTCACTG	129
	GBI.188	CNGCNAAGGAAGATCTCCGAGTTCGGNGTTACTG	130
	GBI.16	AGACCGTAGGG TTCGGGAGCGATAAACAGTCGTT	131
	GBI.126	AGACCGTAGGGGCTTGGGCCA TCAACTGGCGCGG	132
	GBI.114	AGACGGTAGCGCCTTGAGTGAATCAATCAGNAGTAA	133
	GBI.129	AGACCGTTGGGACTATA GGCGAACACCAGCTACCA	134
	GBI.164	AGACGGTAGCCC TTAACGGCGAACAACGCGTTT	135
10	GBI.70	AGACTGT AGAGACTTGATGGGTCCGAACCGTCA	136
	GBI.79	AGACTGT AGAGGCTA GGGTAACAACGGCTCGTTT	137
	GBI.90	AGACTGTGAGAGACTA GGCGAGAAAACGGGGTTCTC	138
	GBI.130	AGACTGT AGAGGCTA GGGCATCAACAGTTCTTCC	139
	GBI.154	AGACTG GAGAGACTA GGCGAGAACCAGGGGCGC	140
	GBI.22	AGAGAGGAGAACTTAT AGGAAACAACGGTCCGGC	141
	GBI.157	AGACTGTAGAGGCTA GGGTAACAACGGCTCGTCTG	142
	GBI.158	AGACTGTTGAGACTAACTGCCAACAAC TGC TGTA	143
	GBI.190	AGAGCTGTTGACACTAACGCGAACAACAAC TGTA	144
15	GBI.66	TGGAGGCGATACTTGGCGAACAACAGGGGCTGTA	145
	GBI.74	ATGCCGAACAACAGTCTGAACAACAGGTC TGTAT	146
	GBI.107	TAGAGCGAATACTTGGCGGAACAACAGGGC TGTA	147
	GBI.178	GGACTGTAGAGACCAGTGGAACAACAGATCG GTA	148
	GBI.118	TGGAGGCGAA TCTGGCGAGACAACAGCTTTATCTC	149
	GBI.137	TGGAGGCGAAGTCTGGCGA ACAAGCGCTTTATCTC	150
	GBI.142	TGGAGGCGAA TCTGTCTGA ACAACAGCTTTATCCC	151
20	GBI.32	GT CGGAGNAACTATGTGTTTTAGAGCCATCCC	152
	GBI.167	GTACGGAGAAAACCTATGTGTTTTAGAGCCATCCC	153
	GBI.184	GTACGGCGCAAACAATGTGTTTTAGAGCNACTCC	154
	GBI.34	GTGTAGACTGCAGAGACTGCCAGTGATCTCTCCC	155
	GBI.45	GTGTAGACTGCAGAGACTGCCAGTGCTCTCTCCC	156
	GBI.72	TGGGGCGAACACAGGTTGAGGCTTACACAGGGTT	157
	GBI.102	AGTAGGCGNACACAGGTTGAGGCTTACACAGGGTT	158
25	GBI.49	GAACAGGCNNN TTACCTCTGTGGCCGTTTATCCCTC	159
	GBI.67	CAGCCCNCTTACCTCTGT GCAGTTTATCCCTCT	160
	GBI.9	AGACATGGACACTAGGGGACACTGCAGCCAACTT	161
	GBI.31	AGACA GGAGTGACTTGGCAGCTNACAGACGCTTC	162
	GBI.95	GAGACA GGACTGACTTGGCAGCTCACAG CGCTTC	163
	GBI.11	TAGTGGCGAACGACAGACTCTCACACACACAGGCTTG	164
	GBI.19	TAAGTGGCGAACGACAG CTCTCACACACA GCCTTG	165
30	GBI.3	TAGTTCCTTGCTTATTCTTGCTTCCCTTGCTG	166
	GBI.5	AGCACTGAGATACGCTTATTCTTGCTCCGGGCTTGT	167
	GBI.15	GAGGACGATCAACAGCGACTTATTCTCACAACTG	168

-42-

	GBI.17	TCCCGCTTATTCTTGTCTCAGCTTATTATTCTTGT	169
	GBI.40	GTGGNNNAATTCTTATTCTTGTCTCTCGTGGT	170
	GBI.50	ACCACTACGATTATTCTTGTCTCCCTGNNTTNNNT	171
	GBI.59	GGTGGTTGAGCTTATTCTTGTCTCGATTTGCACGTGT	172
	GBI.78	ACCTTCCGCTTATTCTTGTCTCGCTTCTTCTTGT	173
	GBI.80	AGTTGTGTCCGCGTTTCTTGTCTCCCTTTTCCT	174
	GBI.81	TAGTCCCTTGCTTATTCTTGTCTTCCCTTGTCTG	175
5	GBI.82	ACCTTCCGCTTATTCTTGTCTCTGCTTATTCTTGT	176
	GBI.85	GTCGCTTATTCTTGTCTCCCTCTTATTCTTGTCCC	177
	GBI.103	AGCACGAGATACGCTTATTCTTGTCTCCGCGCTTCT	178
	GBI.108	TGTGTTGTTGTCTTGTCTCATCCCTGTTCTCTC	179
	GBI.111	TAGTGCCTGGGACGCTTATTCTTGTCTCCGGGNGTA	180
	GBI.39	GGAGGCGCTTGTGTCTTGTCTCCCTTGTGTGTCTC	181
	GBI.163	GTGGGGTTGTTGTCTTATTCTTGTCTCCGG	182
	GBI.166	AGTCCCGCTTATTCTTGTCTCCCTTATCGCG	183
	GBI.169	ACACGCTTATTCTTGTCTCCACTTATTCTTGT	184
	GBI.174	GTTGTGCTTATTCTTGTCTCTGTCTGTTTGTGTC	185
10	GBI.177	AGAGTGGGGGGCGCTTATTCTTGTCTCCACTCGCTTGT	186
	GBI.179	GACACCGCGCGCTTATTCTTGTCTCCNNCTTTC	187
	GBI.191	GTTGTGCTTATTCTTGTCTCCCATCCTCTACTC	188
	GBI.180	AGCCGTGTCCAGCTTATTCTTGTCTCCCTNNCTTC	189
	GBI.24	GGTTGTGTGACTTCTATTTGNNTTTCGTGTCCC	190
	GBI.51	GTCGCTGTGTACCGTTTTTTTCTTGTTCCTGTGTC	191
	GBI.71	GGTAGGTCCTTTTCTGTCTTCTTGTCTCTCGC	192
	GBI.77	TGTCTGTCCGTTCTTTTGTCTGTGTTTCCCN	193
	GBI.83	GTACCTGTTGTGAGCTTTTACCCTTCGTTCCCTC	194
15	GBI.87	AGTCCGCTTCTATTTTCACTTCTGTGTGTGTC	195
	GBI.88	GTTGCCGTATCCTTGTGGAGTTTTCGTTTCTCCC	196
	GBI.91	GTTGGTCNGTTCTTTCTCTGTTGTTCTCCTC	197
	GBI.109	TAGTCCCGCGCTTATTTTGTCTCCGTTCCGTT	198
	GBI.115	AGTCCCTCMMNATCCTTTTGTGTCTTGTGTC	199
	GBI.116	TGTGTGTGTGTGCGGTGTTTTTGTCTTCTTCTGTC	200
	GBI.117	GTGTCCGTTGTTCCGCTTTTGTGNCCTGTTTTTCC	201
	GBI.133	AGAAGCCTTGTGCTCTTCCGTTTCTTCTTGTGTC	202
	GBI.186	ACCGGTAGGAGTCCGTTTTTGTGTTGCACTATGCC	203
	GBI.175	ACCCNACTGTGATGTTGCTGTTTTGTTTCTCCNC	204
20	GBI.20	GGTCACACCAGTCACAGCACCTACGTCTTCCCTC	205
	GBI.21	GTAGTGGAACCGACTAGCGGGGTGAAGACTCCTC	206
	GBI.25	TAGCCACAGCAATTTTAGTCTGAGTTCCGTC	207
	GBI.30	AGGCTGCCGTAAGCTTTGGGAATTGGCTTGTGTC	208
	GBI.53	TGGAGGCGAATCTGGCGAACAACAGCCTTATCTC	209
	GBI.54	GAGGCTGTAGAGGCTGACTGCGCGCAGCTGCTGTG	210
	GBI.57	GAGGCGAGACAGGGTAGCACCTCACAACATGC	211
	GBI.65	TGGACTGGAGAGACCTTAGGAGTCATAACTCTCTC	212
	GBI.98	GACTGAAGAGCTCAGAGGCGATACAGGCCGCTGT	213
25	GBI.106	AAGACAGCAGTGGCTAGGGCGATAACTGTCAACAC	214
	GBI.110	GACCGCAGGGTTCCGGAGCGATAAACTAGACCTT	215
	GBI.112	CATGCGGGTTTGTCCGACCTCAGCAACAGCTAC	216
	GBI.113	GAAGGCGNANACAGGAGGAAAGCTNACACCTATC	217
	GBI.121	GACTGTAGAGACAGGACGTACAATAGGCTCACTC	218
	GBI.122	GTTGCATTCCAGGACCGTTCTGTCTNGTACCTCGCGC	219
	GBI.127	ATGGGGGCGAACCCTTTGCGCTCACAACCTACCTGC	220
	GBI.128	GAACGACGGGACAGGGCTGAAAACAGGCAGCTAC	221
	GBI.131	TGCGCGGTGTTGCNCTTTGTTCTATTCTCTGTGTC	222
	GBI.135	TGAACCACAAGCCCCAACTAACAACACCTGTC	223
30	GBI.143	AGGGTGAGATCCAGGGCGCGCTACGTGCGGTGTC	224
	GBI.147	ACCGCGACTCTTTGCGTACTTCTTGGTCTTCCGCCT	225
	GBI.151	TGGGCGAAGGGTCTTGGACGAGGACAGGCGC	226
	GBI.165	AGGTACCCGTTATCTCTTCTGTTGCTCTTTCGC	227

-43-

5	GBI.168	AGTCAAACCCCTCTACGCTGTTGTTGATGTCTCCC	228
	GBI.172	TAGGCAGAACTCACTAAAAGGTCCAAC TGGTTCC	229
	GBI.173	TGGACAGGACTCACCTACAAGGCTTACAACGCAT	230
	GBI.176	GTAGACTGTAGAGTTACGGCGCGACTACAACGCT	231
	GBI.192	AGGCGGTAGCTACTAACATATCACAACATCTTAC	232

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-44-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: JENSEN, KIRK
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- (ii) TITLE OF INVENTION: SYSTEMATIC EVOLUTION OF LIGANDS
BY EXPONENTIAL ENRICYMENT:
TISSUE SELEX
- (iii) NUMBER OF SEQUENCES: 240
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette, 3 1/2 diskette, 1.44 MB
 - (B) COMPUTER: IBM pc compatible
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:
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 - (A) APPLICATION NUMBER: 08/434,425
 - (B) FILING DATE: 05-MAY-1995
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/437,667
 - (B) FILING DATE: 05-MAY-1995
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/434,001
 - (B) FILING DATE: 05-MAY-1995
- (vii) PRIOR APPLICATION DATA:

-45-

- (A) APPLICATION NUMBER: 08/433,585
(B) FILING DATE: 05-MAY-1995
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Barry J. Swanson
(B) REGISTRATION NUMBER: 33,215
(C) REFERENCE/DOCKET NUMBER: NEX30/PCT
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 793-3333
(B) TELEFAX: (303) 793-3433
- (2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
GGGAGCTCAG AATAACGCT CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNTTCGACA TGAGGCCCGG ATC 73
- (2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GGGAGCTCAG AATAACGCT CAA 23
- (2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: NOTE: N at positions 1 represents
three biotins
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
NGATCCGGGC CTCATGTCGA A 21
- (2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-46-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
AACTCAGTGG TAGGTAACGG TT 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GGGAGCTCAG AATAAACGCT CAACTCAGTG GTAGGTAACG GTTCAAGACG 50
GGATTGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
GGGAGCTCAG AATAAACGCT CAACTCAGTG GTAGGTAACG GTTATATCCG 50
GAATTGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GGGAGCTCAG AATAAACGCT CAAACTCAG TATAAGGTAA CGGTTCCAAC 50
CCAGATTCGA CATGAGGCCG GGATC 75

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCCAAGG TAACGGTTCC 50
CTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs

-47-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCTAGGT AACGGTTCCTC 50
TTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGAGCTCAG AATAAACGCT CAAACTCAGT AATGCACCAG TAACGGTTAC 50
ATCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGAGCTCAG AATAAACGCT CAACTCAGTA GCAAGGTAAC GGTTCAGATC 50
CACTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGAGCTCAG AATAAACGCT CAAGTCATAA CGGTTAGCCA GAGGACCGTG 50
CCTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGAGCTCAG AATAAACGCT CAACAGGTCG ATCGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

-48-

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGGAGCTCAG AATAAACGCT CAAGAGGTCG ATCGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGAGCTCAG AATAAACGCT CAACAGGTCG ATTGAGTCAG GTAGGCGCCG 50
AGATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGAGCTCAG AATAAACGCT CAAGGCGTGT CGATGTGGAA TCACAACCTG 50
TCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGAGCTCAG AATAAACGCT CAAGGTTGTC GACGCATTAT AGCGACATCG 50
TCTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-49-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
GGGAGCTCAG AATAAACGCT CAAGTGGAGT CGACACGCTG TGACCTTTGG 50
CATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GGGAGCTCAG AATAAACGCT CAAGTGAGTC GACACGCCGC GACCTTTGGT 50
ATTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GGGAGCTCAG AATAAACGCT CAAGTGCCTC GAGGCATTGC AACCTTTGGT 50
CTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GGGAGCTCAG AATAAACGCT CAATAGACCG TCGATGCTTG CAACTTTACG 50
TATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
GGGAGCTCAG AATAAACGCT CAATAGTTGC CCACCGTTGT CCAATTGATC 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

-50-

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGAGCTCAG AATAAACGCT CAATGGTTGC CCATCGTTGT CCAATTGATC 50
GTTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGAGCTCAG AATAAACGCT CAATGTTGCC CATTCGTCGT CCAAGTGAAC 50
GTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGAGCTCAG AATAAACGCT CAATGAATTG CCAACGTCG CCCGAATGAT 50
GCGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGAGCTCAG AATAAACGCT CAAAGGCGGT GTTACTTCTC ACGAATTGAG 50
GAAGTTCGAC ATGAGGCCCG GATC 74

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGGAGCTCAG AATAAACGCT CAAAGCGTTG TTA CTCTCA CGAATTGAGG 50

-51-

AAGTTCGACA TGAGGCCCGG ATC

73

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGGAGCTCAG AATAAACGCT CAATGAGAGG GGCAACCTTG AGTCTTTCAT 50
GCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGGAGCTCAG AATAAACGCT CAAAGCAGCG GGCAACCTTG AGTATTTCAT 50
GCTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGGAGCTCAG AATAAACGCT CAAACCCGGG CAACCGTTTCG GTCTTTCAGT 50
CTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGGAGCTCAG AATAAACGCT CAACATCGTT GACACCCTCG TGTGCTTCAG 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid

-52-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGGAGCTCAG AATAAACGCT CAACATCGCT TGACAGCTGT GCTGCTTCAG 50
TTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGGAGCTCAG AATAAACGCT CAAGGGTGAT CGAAGCCTAG GTGAGCTTGA 50
GCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGAGCTCAG AATAAACGCT CAAGGGTGTC CGAGCATCCG TAGCTTGAGT 50
CGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGAGCTCAG AATAAACGCT CAAACGAATC GCATTGCCCA ACGTTGCCCA 50
AGATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGGAGCTCAG AATAAACGCT CAACCGAATC GCATTGCCCA ACGTTGCCCA 50
AGATTTCGACA TGAGGCCCGG ATC 73

-53-

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGGAGCTCAG AATAAACGCT CAATGTCGGA TAAGTCGCCC AACGTTGCCC 50
ATTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGAGCTCAG AATAAACGCT CAAGTGGAGC GATTCGCGAA AATCGACTTG 50
CATTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGGAGCTCAG AATAAACGCT CAACTGGAGC GATTCGGAAA ATCGACTTGC 50
ATTTTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGGAGCTCAG AATAAACGCT CAACATCTGG ATGTTCAACC TTCTGGTCTT 50
GCGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-54-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GGGAGCTCAG AATAAACGCT CAACTACCCG GTTGAACCTT CGCTCTTGCG 50
TAGTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
GGGAGCTCAG AATAAACGCT CAATGCTCCC CGAAACCCTA TTTCTTGCTG 50
CTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
GGGAGCTCAG AATAAACGCT CAATGCACCT CACCTCCTTA CACTTTCCTT 50
CTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
GGGAGCTCAG AATAAACGCT CAAACCTCGT ACTGCCATCT CTCCCCTCAT 50
GTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
GGGAGCTCAG AATAAACGCT CAAACACTCA CGACTTTTCA TCTTTCTCCT 50
TCTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

-55-

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGGAGCTCAG AATAAACGCT CAAAACCCTT CTTCACTCTT CTCGCTCTCC 50
TTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGGAGCTCAG AATAAACGCT CAACCCTTCC AATTCCTCTT ACTCCTCTCT 50
CCTTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGAGCTCAG AATAAACGCT CAAGCACTTC TCACTATTCC TTCCTTCTCT 50
CTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGAGCTCAG AATAAACGCT CAAACCCTAC TCTCCACTCA CATCTTCTTC 50
CCCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGGAGCTCAG AATAAACGCT CAATACCTCA CACTCTCTTA ATCTCTTCTC 50

-56-

TTCTTCGACA TGAGGCCCGG ATC

73

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGGAGCTCAG AATAAACGCT CAACGGTTCA TCTTTTCTTG TTATTTTCC
ACTATTCGAC ATGAGGCCCGG GATC

50

74

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GGGAGCTCAG AATAAACGCT CAAGTGGCCT CAACTGCTA GGAGTAAACA
TGTTTCGACA TGAGGCCCGG ATC

50

73

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GGGAGCTCAG AATAAACGCT CAATAGGGGT AGGGCGCAAT ATTCACCGGG
CCTTCGACAT GAGGCCCGGA TC

50

72

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GGGAGCTCAG AATAAACGCT CAAGGAGCGC GATACGTTTA CTTCTGATCA
TGTTTCGACAT GAGGCCCGGA TC

50

72

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid

-57-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GGGAGCTCAG AATAAACGCT CAAAGAGGAG TCTTGCTGTC CGTACACAGC 50
TTATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGGAGCTCAG AATAAACGCT CAATCCCTTG AACCATCGGT CTTGCGTTCC 50
ATGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GGGAGCTCAG AATAAACGCT CAAACAAGAG GGTCTTGCCG CACCATTTCGG 50
CTATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGGAGCTCAG AATAAACGCT CAAACGAGTT ACAGCCACCC ATGCTGTCCG 50
TGATTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGGAGCTCAG AATAAACGCT CAAGACAGCG TGATTCTCTCC GCTCTGCTGC 50
TATTTCGACA TGAGGCCCGG ATC 73

-58-

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGGAGCTCAG AATAAACGCT CAACGGGACC TTGAGTATTC CTCATTATCG 50
TTCTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGGAGCTCAG AATAAACGCT CAAGTAGTGA AGCTCGTACA GAGGTATTGC 50
GTATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGGAGCTCAG AATAAACGCT CAAAGCCGAA TTAGTAGCGT ATAGCGTGTT 50
GTGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGGAGCTCAG AATAAACGCT CAAGGGCAAT ACACAACACT CTACCTCACC 50
TCATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-59-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGGAGCTCAG AATAAACGCT CAATCAGAGA TTCTTCCCGG CTATCCCGGG 50
TGATTGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GGGAGCTCAG AATAAACGCT CAATAGGCCG GGTGAGCTAC TTCTAGTAGG 50
GTGTTGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GGGAGCTCAG AATAAACGCT CAAGTTGTGA TCCATTAGCG GCACCGCCTC 50
CATTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGGAGCTCAG AATAAACGCT CAATCCGGAA AGCAACGCAT ACTTCGCATG 50
TCGTTGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GGGAGCTCAG AATAAACGCT CAAGTGAGCG TACCGGAGTG TGTTACCAAT 50
TATTCGACAT GAGGCCCGGA TC 72

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

-60-

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGGAGCTCAG AATAAACGCT CAACACATCT GCAGACTGTA CCCCACATGG 50
CAATTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GGGAGCTCAG AATAAACGCT CAAGAGGGCC GGGTTAGCCT TTAAAGGTTG 50
TGTTTCGACA TGAGGCCCGG ATC 73

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GCCTGTTGTG AGCCTCCTNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNGCCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 1 is fluroscein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

NGCCTGTTGT GAGCCTCCT 19

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

-61-

GGGAGACAAG AATAAGCG

18

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGGCTTT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGGCTTT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGAGCTTT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGGC CAGGGGCGAT AACCGCACTT 50
TCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid

-62-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GCCTGTTGTG AGCCTCCTGG CTGCTGAGTC CAGGGGCGAT AACGGCCTTT 50
CCGCTTATTCT TGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GCCTGTTGTG AGCCTCCTTA GCGAACACAG GGNCCACAA CTGGCTATCT 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GCCTGTTGTG AGCCTCCTTA GCAGAACACA GGGGNCCACA ACTGGCTATC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GCCTGTTGTG AGCCTCCTTA GGCGAACACA GGGGTCCACA ACTGGCTATC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GCCTGTTGTG AGCCTCCTTA GCGAACACAG GGTCAACAGC TCACACGGCC 50
CGCTTATTCT TGTCTCCC 68

-63-

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCCTGTTGTG AGCCTCCTTA GCGAACGARC GGTGCCCTGC TCTCAACTGG	50
TTTCGCTTAT TCTTGTCTCC C	71

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GCCTGTTGTG AGCCTCCTTA GGCCGGAGGG ACTAATAGCT TACAGCGCAC	50
TACGCTTATT CTTGTCTCCC	70

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GCCTGTTGTG AGCCTCCTTA GGCCGGAGGG ACTAATAGCT TACAAGGCAC	50
TACGCTTATT CTTGTCTCCC	70

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GCCTGTTGTG AGCCTCCTTA GGAGCGCGAA CAACGGGGGA GGTCTCACAC	50
TGCGCTTATT CTTGTCTCCC	70

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-64-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:
GCCTGTTGTG AGCCTCCTTA GGGGNGNNA TACAACAGGT CGGTCACAAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:
GCCTGTTGTG AGCCTCCTTA GGGCGGAGNG NGGCGGTCAT CCTGGNNACA 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:
GCCTGTTGTG AGCCTCCTAG GCAGAAGTGA GCTTGGGCTC GCAACTCTCT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:
GCCTGTTGTG AGCCTCCTAG GCNGTAGGNG CTAGGGNGNA CTCGTATTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:
GCCTGTTGTG AGCCTCCTAG GCAGCAGTGA CTTGGACGAC AACAGCTATG 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

-65-

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GCCTGTTGTG AGCCTCCTAG GCAGTAGTGA CTTGGGCGCA GAGGAGGGTA 50
GTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GCCTGTTGTG AGCCTCCTAG GGCGCAGGGT CTAGGGCANC CAACAGCTAT 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

GCCTGTTGTG AGCCTCCTAG GCGAAGGGNC TAGGGTGNAC AGCAGCGGTG 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTTAGGTT CGGTTACAGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTAGGTTT GGTTCACGTC 50

-66-

CCGCTTATTC TTGTCTCCC

69

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GCCTGTTGTG AGCCTCCTCC CAGAGGGAAG ACTTTAGGTT CGGTTACGT 50
CCCCGCTTAT TCTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GCCTGTTGTG AGCCTCCTNC CAGAGGNAG ACTTTAGGTT CGGTTACGT 50
CCCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG GCTTTAGGTT CGGTTACGT 50
CCCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GCCTGTTGTG AGCCTCCTNN NAGAGGGAAG ACTTTAGGTT CGGTTACGT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid

-67-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GCCTGTTGTG AGCCTCCTNN NAGAGGGNAG ACTTTAGGTT CGGTTCACGT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GCCTGTTGTG AGCCTCCTGT GTGCAACAGA GCAGNNNTTG TCTAACATCA 50
CTTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GCCTGTTGTG AGCCTCCTGG GGCGAACAGC AGCTACTCAC AACATGTCCG 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GCCTGTTGTG AGCCTCCTGT GGCGAACACG GGTCAAGGGC TTCACAATCT 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GCCTGTTGTG AGCCTCCTAT GGCGAACACA GCAACTCGCT CCAACTCTC 50
TCCGCTTAT TCTTGTCTCC C 71

-68-

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GCCTGTTGTG AGCCTCCTGT AGGCGAACAC AGGTTGAGGC TTACACAGGG 50
NTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GCCTGTTGTG AGCCTCCTAG CGAACAACACTG ACTGACGGCA GGGTCAACAC 50
NNCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GCCTGTTGTG AGCCTCCTTA CGAACAACAG CATTACACA GGCCTTTTTG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GCCTGTTGTG AGCCTCCTAG CGAGCAACAT CTTTCGCAAC AGGTTTGGTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-69-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:
GCCTGTTGTG AGCCTCCTTT GCGAACACA GCAACTCGCT CACAACTATC 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:
GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG TTGGTGGAGG CGAACGTACC 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:
GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG TTGGTGGAGG CGAACGTCCT 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:
GCCTGTTGTG AGCCTCCTAG GTTGGGTAGG CTGGTGGAGG CGNACGTCCC 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:
GCCTGTTGTG AGCCTCCTAG GTTCGCAGGC TGGCTGGAGG CGCGCGACCC 50
AACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:

-70-

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

GCCTGTTGTG AGCCTCCTGG TTTGACCGTA ACAATTGTTA AAGCTCCGGG 50
NNCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAATTGTTA AAGCTCCGGG 50
NCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

GCCTGTTGTG AGCCTCCTGG TTTGATCTCT AACAAATTGTT AAAGCTCCAG 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GCCTGTTGTG AGCCTCCTGG TCTGATCGCT AACAAATTGTT AAAGCTCCGG 50
GGCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAAATTGTT AAAAGCTCCG 50

-71-

GGCCCGCTTA TTCTTGTCTC CC

72

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

GCCTGTTGTG AGCCTCCTGG TTTGTCGTAA CAATTGTAA AGCTCCGGGA 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAGTTGTAA AAAGCTCCGG 50
GCGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GCCTGTTGTG AGCCTCCTGG TCTGATCGTA ACAATTGTAA AGCTCCGGGC 50
GCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

GCCTGTTGTG AGCCTCCTCC GCCAAGGGAG CTCTCCGAGC TCGGCGCCAC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid

-72-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GCCTGTTGTG AGCCTCCTNC NNCNAAGGAA GATCTCCGAG TCGGCGTCA 50
CTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

GCCTGTTGTG AGCCTCCTCT GCCGGGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

GCCTGTTGTG AGCCTCCTCC GCCAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

GCCTGTTGTG AGCCTCCTCN GCNAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

GCCTGTTGTG AGCCTCCTCN GCCAAGGAAG ATCTCCGAGT TCGGCGTCAC 50
TACGCTTATT CTTGTCTCCC 70

-73-

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

GCCTGTTGTG AGCCTCCTCN NCNAAGGAAG ATCTCCAGTT CGGCGTCACT 50
GCGCTTATT CTTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

GCCTGTTGTG AGCCTCCTCN GCNAAGGAAG ATCTCCGAGT TCGGNGTTAC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

GCCTGTTGTG AGCCTCCTAG ACCGTAGGGT TCGGGAGCGA TAAACAGTCG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

GCCTGTTGTG AGCCTCCTAG ACCGTAGGGG CTTGGGCCAT CAACTGGCGC 50
GGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-74-

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:
GCCTGTTGTG AGCCTCCTAG ACGGTAGCGC CTTGAGTGAA TCAATCAGNA 50
GTAACGCTTA TTCTTGTCTC CC 72
- (2) INFORMATION FOR SEQ ID NO:134:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:
GCCTGTTGTG AGCCTCCTAG ACCGTTGGGA CTATAGGCGA ACACCAGCTA 50
CCACGCTTAT TCTTGTCTCC C 71
- (2) INFORMATION FOR SEQ ID NO:135:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:
GCCTGTTGTG AGCCTCCTAG ACGGTAGCCC TTAACGGCGA ACAACGCGTT 50
TCGCTTATTC TTGTCTCCC 69
- (2) INFORMATION FOR SEQ ID NO:136:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:
GCCTGTTGTG AGCCTCCTAG ACTGTAGAGA CTTGATGGGT CGCAACCGTC 50
ACGCTTATTC TTGTCTCCC 69
- (2) INFORMATION FOR SEQ ID NO:137:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:
GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGTAAC AACGGCTCGT 50
TTGCTTATT CTTGTCTCCC 70
- (2) INFORMATION FOR SEQ ID NO:138:
(i) SEQUENCE CHARACTERISTICS:

-75-

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

GCCTGTTGTG AGCCTCCTAG ACTGTGAGAG ACTAGGCGAG AAACGGGGTT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGCATC AACAGTTCTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

GCCTGTTGTG AGCCTCCTAG ACTGGAGAGA CTAGGCGAGA ACCGGGGCGC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

GCCTGTTGTG AGCCTCCTAG AGAGGAGAAC TTATAGGAAA CAACGGTCGG 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

GCCTGTTGTG AGCCTCCTAG ACTGTAGAGG CTAGGGTAAC AACGGCTCGT 50

-76-

CTGCGCTTAT TCTTGTCTCC C

71

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GCCTGTTGTG AGCCTCCTAG ACTGTTGAGA CTAAGTGC GA ACAACTGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

GCCTGTTGTG AGCCTCCTAG AGCTGTTGAC ACTAACGCGA ACAACAAGT 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GCCTGTTGTG AGCCTCCTTG GAGGCGATAC TTGGCGAACA ACAGGGGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

GCCTGTTGTG AGCCTCCTAT GCCGAACAAC AGTCTGAACA ACAGGTCTGT 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid

-77-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

GCCTGTTGTG AGCCTCCTTA GAGCGAATAC TTGGCGGAAC AACAGGGCTG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

GCCTGTTGTG AGCCTCCTGG ACTGTAGAGA CCAGTGGAAC AACAGATCGG 50
TACGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGGCGAGACA ACAGCTTTAT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

GCCTGTTGTG AGCCTCCTTG GAGGCGAAGT CTGGCGAACA AGCGCTTTAT 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGTCGAACAA CACGTTTATC 50
CCCGCTTATT CTTGTCTCCC 70

-78-

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

GCCTGTTGTG AGCCTCCTGT CGGAGNAAAC TATGTGTTTT AGAGCCATCC 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

GCCTGTTGTG AGCCTCCTGT ACGGAGAAAA CTATGTGTTT TAGAGCCATC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

GCCTGTTGTG AGCCTCCTGT ACGGCGCAAA CAATGTGTTT TAGAGCNACT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:155:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

GCCTGTTGTG AGCCTCCTGT GTAGACTGCA GAGACTGCCA GTGATCTCTC 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:156:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-79-

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:
GCCTGTTGTG AGCCTCCTGT GTAGACTGCA GAGACTGCCA GTGCTCTCTC 50
CCCGCTTATT CTTGTCTCCC 70
- (2) INFORMATION FOR SEQ ID NO:157:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:
GCCTGTTGTG AGCCTCCTTT GGGGCGAACA CAGGTTGAGG CTTACACAGG 50
GTTTCGCTTAT TCTTGTCTCC C 71
- (2) INFORMATION FOR SEQ ID NO:158:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:
GCCTGTTGTG AGCCTCCTAG TAGGCGNACA CAGGTTGAGG CTTACACAGG 50
GTTTCGCTTAT TCTTGTCTCC C 71
- (2) INFORMATION FOR SEQ ID NO:159:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:
GCCTGTTGTG AGCCTCCTGA ACAGGCNNNT TACCTCTGTG GCCGTTTATC 50
CCTCCGCTTA TTCTTGTCTC CC 72
- (2) INFORMATION FOR SEQ ID NO:160:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:
GCCTGTTGTG AGCCTCCTCA GCCCNCCTTA CCTCTGTGCA GTTTATCCCT 50
CTCGCTTATT CTTGTCTCCC 70
- (2) INFORMATION FOR SEQ ID NO:161:
(i) SEQUENCE CHARACTERISTICS:

-80-

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

GCCTGTTGTG AGCCTCCTAG ACATGGACAC TAGGGGACAC TGCAGCCAAC 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:162:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

GCCTGTTGTG AGCCTCCTAG ACAGGAGTGA CTTGGCAGCT NACAGACGCT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:163:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

GCCTGTTGTG AGCCTCCTGA GACAGGACTG ACTTGGCAGC TCACAGCGCT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:164:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

GCCTGTTGTG AGCCTCCTTA GTGGCGAACG ACAGACTCTC ACACACACAG 50
GCTTGCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

GCCTGTTGTG AGCCTCCTTA AGTGGCGAAC GACAGCTCTC ACACACAGGC 50

-81-

TTGCGCTTAT TCTTGTCTCC C

71

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

GCCTGTTGTG AGCCTCCTTA GTTCCTTGCT TATTCTTGCT TCCCTTGTCT
GCGCTTATTC TTGTCTCCC

50

69

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

GCCTGTTGTG AGCCTCCTAG CACTGAGATA CGCTTATTCT TGTCTCCGGG
CTTGTCGCTT ATTCTTGTCT CCC

50

73

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

GCCTGTTGTG AGCCTCCTGA GGACGATCAA CAGCGACTTA TTCTCACAAC
TGCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

GCCTGTTGTG AGCCTCCTTC CCGCTTATTC TTGTCTCAGC TTATTATTCT
TGTCGCTTAT TCTTGTCTCC C

50

71

(2) INFORMATION FOR SEQ ID NO:170:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid

-82-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

GCCTGTTGTG AGCCTCCTGT GGNNAATT CNCTTATTCT TGTCTCTCGT 50
GGTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:171:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

GCCTGTTGTG AGCCTCCTAC CAGTACGATT ATTCTTGTCT CCCTGNNTTN 50
NNTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:172:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

GCCTGTTGTG AGCCTCCTGG TGGTTGAGCT TATTCTTGTC TCGATTTGCA 50
CGTGTCGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:173:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

GCCTGTTGTG AGCCTCCTAC CTTGCGGCTT ATTCTTGTCT CGCTTCTTCT 50
TGTCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:174:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

GCCTGTTGTG AGCCTCCTAG TTGTTGTCCG CGTTTCTTGT CTCCTTTTC 50
CTCGCTTATT CTTGTCTCCC 70

-83-

(2) INFORMATION FOR SEQ ID NO:175:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

GCCTGTTGTG AGCCTCCTTA GTCCCTTGCT TATTCTTGTC TTCCCTTGTC 50
TGCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:176:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

GCCTGTTGTG AGCCTCCTAC CTTCCGGCTT ATTCTTGTTT TCTGCTTATT 50
CTTGTCGCTT ATTCTTGTTT CCC 73

(2) INFORMATION FOR SEQ ID NO:177:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

GCCTGTTGTG AGCCTCCTGT CGCTTATTCT TGTCTCCCTC TTATTCTTGT 50
CCCCGCTTAT TCTTGTTCTC C 71

(2) INFORMATION FOR SEQ ID NO:178:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

GCCTGTTGTG AGCCTCCTAG CACGAGATAC GCTTATTCTT GTCTCCGCGC 50
TTCTCGCTTA TTCTTGTTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:179:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-84-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:
GCCTGTTGTG AGCCTCCTTG TGTGTTGTT CTTTGTGTCA TCCCTGTTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:180:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:
GCCTGTTGTG AGCCTCCTTA GTGCCTGGGA CGTTATTCT TGTCTCCGGG 50
GNCTACGCTT ATTCTTGTCT CCC 73

(2) INFORMATION FOR SEQ ID NO:181:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:
GCCTGTTGTG AGCCTCCTGG AGGCGCTTGT GTCTTGTTC CTTGTGTGTC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:182:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:
GCCTGTTGTG AGCCTCCTGT GGGGTTGTTG TCTTATTCTT GTCTCCGGCG 50
CTTATTCTTG TCTCCC 66

(2) INFORMATION FOR SEQ ID NO:183:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:
GCCTGTTGTG AGCCTCCTAG TCCCCGCTTA TTCTTGTCTC CCTTATCGCG 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:184:

(i) SEQUENCE CHARACTERISTICS:

-85-

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

GCCTGTTGTG AGCCTCCTAC ACGCTTATTC TTGTCTCCAC TTATTCTTGT 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:185:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

GCCTGTTGTG AGCCTCCTGT TGTCGCTTAT TCTTGTCTCT GTCTGTTTGT 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:186:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

GCCTGTTGTG AGCCTCCTAG AGTGGGGGGC GCTTATTCTT GTCTCCACTC 50
GCTTGTCGCT TATTCTTGTC TCCC 74

(2) INFORMATION FOR SEQ ID NO:187:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

GCCTGTTGTG AGCCTCCTGA CACCCGCCGC GCTTATTGTT GTCTCCNNNC 50
TTCCGCTTA TTCTTGCTC CC 72

(2) INFORMATION FOR SEQ ID NO:188:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

GCCTGTTGTG AGCCTCCTGT TGTCGCTTAT TCTTGTCTCC CATCCTCTAC 50

-86-

TCCGCTTATT CTTGTCTCCC

70

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

GCCTGTTGTG AGCCTCCTAG CCGTGTCAG CTTATTCTTG TCTCCTNNCT
TCCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

GCCTGTTGTG AGCCTCCTGG TTGTGTGACT TCTATTTGNN TTTCGTGTCC
CCGCTTATTC TTGTCTCCC

50

69

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

GCCTGTTGTG AGCCTCCTGT CGCTGTGTAC CGTTTTTTTC TTGTTGCCT
GTCCGCTTAT TCTTGTCTCC C

50

71

(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

GCCTGTTGTG AGCCTCCTGG TAGGTCCTTT TCTGTCTTCC TTGTTCTCTC
GCCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:193:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid

-87-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

GCCTGTTGTG AGCCTCCTTG TCTGTCCGT CTTTTGTCT GTGTTTCCC 50
NCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:194:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

GCCTGTTGTG AGCCTCCTGT ACCTGTTGTC AGCTTTTACC CTTGTTTCT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:195:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

GCCTGTTGTG AGCCTCCTAG TCGGATTCT ATTTTCACT TTCTGTTGTT 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:196:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

GCCTGTTGTG AGCCTCCTGT TGCCGTATCC TTGTGGAGTT TTCGTTTCTC 50
CCCCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:197:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

GCCTGTTGTG AGCCTCCTGT TGGTCNGTTC CTTTCTCTGT TGTTCTCCTC 50
CGCTTATTCT TGTCTCCC 68

-88-

(2) INFORMATION FOR SEQ ID NO:198:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

GCCTGTTGTG AGCCTCCTTA GTCCCGCGGC TTATTTTGT CTCCGTTCCG 50
TTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:199:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

GCCTGTTGTG AGCCTCCTAG TCCCTCNNNN ATCCTTTTGT TGTCTTGCTG 50
TCCGCTTATT CTTGTCTCCC 70

2) INFORMATION FOR SEQ ID NO:200:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

GCCTGTTGTG AGCCTCCTTG TGTGTGTGTC GGTGGTTTTT TGTCTTCCTT 50
TTGCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:201:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

GCCTGTTGTG AGCCTCCTGT GTCCGTTGTT CGCGTTTTGT GNCCTGTTTT 50
TCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:202:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-89-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:
GCCTGTTGTG AGCCTCCTAG AAGCCTTGTC GTCTTCCGT TTCTTCTTGT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:203:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:
GCCTGTTGTG AGCCTCCTAC CGGTAGGAGT CCGTTTTTGT TTGCACTATG 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:204:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:
GCCTGTTGTG AGCCTCCTAC CCNACTGTGA TGTCGTGTT TTGTTCTCCTCC 50
NCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:205:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:
GCCTGTTGTG AGCCTCCTGG TCACACCAGT CACAGCACCT ACGTCCTGCC 50
CTCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:206:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:
GCCTGTTGTG AGCCTCCTGT AGTGGAACCG ACTAGCGGGG TGAAGACTCC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:207:

(i) SEQUENCE CHARACTERISTICS:

-90-

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

GCCTGTTGTG AGCCTCCTTA GCCACAGCA ATTTAGTCT GAGTTCCGTC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:208:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

GCCTGTTGTG AGCCTCCTAG GCTGCCGTAA GCTTTGGGAA TTGGCCTGCT 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:209:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

GCCTGTTGTG AGCCTCCTTG GAGGCGAATC TGGCGAACAA CAGCCTTATC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:210:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

GCCTGTTGTG AGCCTCCTGA GGCTGTAGAG GCTGACTGCG CGCAGCTGCT 50
GTGCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:211:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

GCCTGTTGTG AGCCTCCTGA GGCGAGACAG GGTAGCACCT CACAACATGC 50

-91-

CGCTTATTCT TGTCTCCC

68

(2) INFORMATION FOR SEQ ID NO:212:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

GCCTGTTGTG AGCCTCCTTG GACTGGAGAG ACCTTAGGAG TCATAACTCT
CTCCGCTTAT TCTTGTCTCC C

50

71

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

GCCTGTTGTG AGCCTCCTGA CTGAAGAGCT CAGAGGCGAT ACAGGCCGCT
GTCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

GCCTGTTGTG AGCCTCCTAA GACAGCAGTG GCTAGGGCGA TAACTGTCAC
CACCGCTTAT TCTTGTCTCC C

50

71

(2) INFORMATION FOR SEQ ID NO:215:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

GCCTGTTGTG AGCCTCCTGA CCGCAGGGTT CGGGAGCGAT AAAC TAGACC
TTCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:216:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid

-92-

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

GCCTGTTGTG AGCCTCCTCA TCGGGTTTG TCCGGACCTC AGCAACAGCT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:217:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

GCCTGTTGTG AGCCTCCTGA AGGCGNANAC AGGAGGAAAG GCTNACACCT 50
ATCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:218:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

GCCTGTTGTG AGCCTCCTGA CTGTAGAGAC AGGACGTACA ATAGGCTCAC 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:219:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

GCCTGTTGTG AGCCTCCTGT TGCATTCCAG GACCGTTCTG TCNGTACCTC 50
GCGCCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:220:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

GCCTGTTGTG AGCCTCCTAT GGGGGCGAAC CTTTGCCTC ACAACCTACC 50
TGCCGCTTAT TCTTGTCTCC C 71

-93-

(2) INFORMATION FOR SEQ ID NO:221:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

GCCTGTTGTG AGCCTCCTGA ACGACGGGAC AGGGCTGAAA ACAGGCAGCT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:222:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

GCCTGTTGTG AGCCTCCTTG CGCGGTGTTG CNCTTTGTTC TATTCTCCTG 50
TCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:223:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

GCCTGTTGTG AGCCTCCTTG AACCACAAGC CCAACTAAC AACACCCTGC 50
CGCTTATTCT TGTCTCCC 68

(2) INFORMATION FOR SEQ ID NO:224:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

GCCTGTTGTG AGCCTCCTAG GGTGAGATCC AGGGCGCGCT ACGTGCGTGT 50
CCGCTTATTC TTGTCTCCC 69

(2) INFORMATION FOR SEQ ID NO:225:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-94-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:
GCCTGTTGTG AGCCTCCTAC CGCGACTCTT TGCCTACTTC TTGGTCTTCC 50
GCCTCGCTTA TTCTTGTCTC CC 72

(2) INFORMATION FOR SEQ ID NO:226:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 67 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:
GCCTGTTGTG AGCCTCCTTG GCGAAGGGT CTTGGACGAG GACAGGCGCC 50
GCTTATTCTT GTCTCCC 67

(2) INFORMATION FOR SEQ ID NO:227:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:
GCCTGTTGTG AGCCTCCTAG GTCACCGTTA TCTCTTCCTG TTGCTCTTTC 50
GCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:228:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:
GCCTGTTGTG AGCCTCCTAG TCAAACCCCT CTACGCTGTT GTTGATGTCT 50
CCCCGCTTAT TCTTGTCTCC C 71

(2) INFORMATION FOR SEQ ID NO:229:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:
GCCTGTTGTG AGCCTCCTTA GGCAGAACTC ACTAAAAGGT CCAACTGGTT 50
CCCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:230:

(i) SEQUENCE CHARACTERISTICS:

-95-

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

GCCTGTTGTG AGCCTCCTTG GACAGGACTC ACCTACAAGG CTTACAACGC 50
ATCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:231:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

GCCTGTTGTG AGCCTCCTGT AGACTGTAGA GTTACGGCGC GACTACAACG 50
CTCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:232:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:

GCCTGTTGTG AGCCTCCTAG GCGGTAGCTA CTAACATATC ACAACATCTT 50
ACCGCTTATT CTTGTCTCCC 70

(2) INFORMATION FOR SEQ ID NO:233:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) FEATURE:

- (D) OTHER INFORMATION: N at position 1 is fluroscein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:

NGCCTGTTGT GAGCCTCCT 19

(2) INFORMATION FOR SEQ ID NO:234:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:

-96-

GGGAGACAAG AATAAGCG

18

(2) INFORMATION FOR SEQ ID NO:235:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:

GCCTGTTGTG AGCCTCCTNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
NNCGCTTATT CTTGTCTCCC

50

70

(2) INFORMATION FOR SEQ ID NO:236:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:

AACTCAGTAA TGCCAAGGTA ACGGTT

26

(2) INFORMATION FOR SEQ ID NO:237:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:

CGAATCGCAT TGCCCAACGT TGCCCAAGAT TCG

33

(2) INFORMATION FOR SEQ ID NO:238:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:

CGCTCAATAG TTGCCACCG TTGTCCAATT GAGCG

35

(2) INFORMATION FOR SEQ ID NO:239:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:

-97-

GTCGAGGCAT TGCAACCTTT GGTCTTTCGA C

31

(2) INFORMATION FOR SEQ ID NO:240:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:

GGGCAACCTT GAGTATTTCA TGCTTCGACA TGAGGCCCG

39

-98-

CLAIMS:

1. A method for identifying nucleic acid ligands and nucleic acid ligand sequences to a tissue target comprising:

a) preparing a candidate mixture of nucleic acid sequences;

b) contacting said candidate mixture of nucleic acids with said tissue, wherein nucleic acids having an increased affinity to the tissue relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

d) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to said tissue, whereby nucleic acid ligands of said tissue may be identified.

2. The method of Claim 1 further comprising:

e) repeating steps b), c) and d).

3. The method of Claim 1 wherein said tissue is selected from the group consisting of a cell, a subcellular component, an aggregate of cells, a collection of cells, an aggregate of macromolecules.

4. The method of Claim 1 wherein said candidate mixture is comprised of single-stranded nucleic acids.

5. The method of Claim 4 wherein said single-stranded nucleic acids are ribonucleic acids.

6. The method of Claim 4 wherein said single-stranded nucleic acids are deoxyribonucleic acids.

-99-

7. The method of Claim 1 wherein said tissue is selected from the group consisting of red blood cells ghosts, glioblastoma, and lymphoma.

8. A nucleic acid ligand to a tissue target identified according to the method of Claim 1.

9. A purified and isolated non-naturally occurring nucleic acid ligand to tissue.

10. The purified nucleic acid ligand of Claim 9 which is a non-naturally occurring nucleic acid ligand having a specific binding affinity for a tissue target molecule, such target molecule being a three dimensional chemical structure other than a polynucleotide that binds to said nucleic acid ligand through a mechanism which predominantly depends on Watson/Crick base pairing or triple helix binding, wherein said nucleic acid ligand is not a nucleic acid having the known physiological function of being bound by the target molecule.

11. The nucleic acid ligand of Claim 9 which is a deoxyribonucleic acid ligand.

12. The nucleic acid ligand of Claim 9 which is a ribonucleic acid ligand.

13. The nucleic acid ligand of Claim 9 wherein said tissue is selected from the group consisting of a cell, a subcellular component, an aggregate of cells, a collection of cells, an aggregate of macromolecules.

14. The nucleic acid ligand of Claim 13 wherein said subcellular component is a red blood cell ghost.

15. The nucleic acid ligand to a red blood cell ghost of Claim 14 wherein said ligand is a DNA ligand selected from the group consisting of the nucleotide sequences set forth in Table 1, or the corresponding RNA sequences thereof or the corresponding complementary sequences thereof.

-100-

16. The nucleic acid ligand of Claim 15 wherein said ligand is selected from the group consisting of SEQ ID NOS:4-70.

5 17. A purified and isolated non-naturally occurring DNA ligand to a red blood cell ghost, wherein said ligand is substantially homologous to and has substantially the same ability to bind said red blood cell ghost as a ligand selected from the group consisting of the sequences set forth in Table 1 or the corresponding RNA sequences thereof or the corresponding complimentary sequences thereof.

10 18. A purified and isolated non-naturally occurring DNA ligand to a red blood cell ghost, wherein said ligand has substantially the same structure and the same ability to bind said red blood cell ghost as a ligand selected from the group consisting of the sequences set forth in Table 1 or the corresponding RNA sequence thereof or the
15 corresponding complementary sequences thereof.

19. The nucleic acid ligand of Claim 13 wherein said cell is a tumor cell.

20. The nucleic acid ligand of Claim 19 wherein said tumor cell is a glioblastoma.

20 21. The nucleic acid ligand to a glioblastoma of Claim 20 wherein said ligand is a DNA ligand selected from the group consisting of the nucleotide sequences set forth in Table 2, or the corresponding RNA sequences thereof or the corresponding
complementary sequences thereof.

25 22. The nucleic acid ligand of Claim 21 wherein said ligand is selected from the group consisting of SEQ ID NOS:74-232.

30 23. A purified and isolated non-naturally occurring DNA ligand to a glioblastoma, wherein said ligand is substantially homologous to and has substantially the same ability to bind said glioblastoma as a ligand selected from the group consisting of the

-101-

sequences set forth in Table 2 or the corresponding RNA sequences thereof or the corresponding complimentary sequences thereof.

5 24. A purified and isolated non-naturally occurring DNA ligand to glioblastoma, wherein said ligand has substantially the same structure and the same ability to bind said glioblastoma as a ligand selected from the group consisting of the sequences set forth in Table 2 or the corresponding RNA sequence thereof or the corresponding complementary sequences thereof.

10 25. The nucleic acid ligand of Claim 19 wherein said tumor cell is a lymphoma.

26. A method for identifying a macromolecule component of a tissue comprising:

a) identifying a nucleic acid ligand to a new epitope of said macromolecule by the method of Claim 1;

15 b) purifying said macromolecule component of said tissue away from the remainder of said tissue on the basis of affinity between said new epitope and said nucleic acid ligand; and

c) identifying said macromolecule.

20 27. The method of Claim 26 wherein said macromolecule is selected from the group consisting of a protein, lipid and carbohydrate.

28. A purified macromolecule identified according to the method of Claim 26.

25 29. The purified macromolecule of Claim 28 which is selected from the group consisting of a protein, lipid and carbohydrate.

30 30. The purified macromolecule of Claim 29 which is a tumor associated antigen.

30

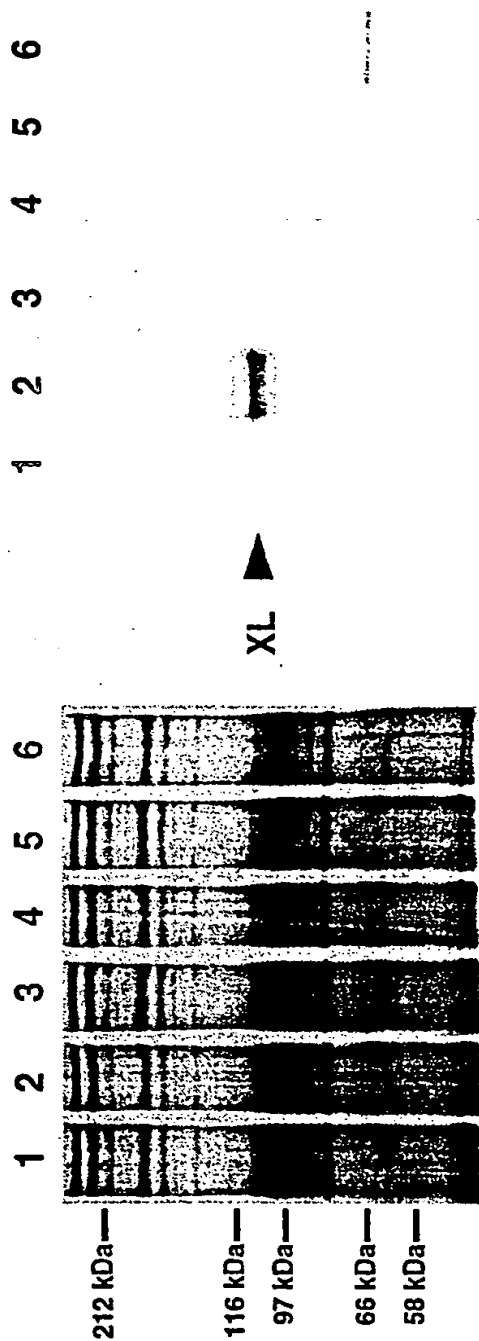


Figure 1

crosslinking of truncate ligand c56t

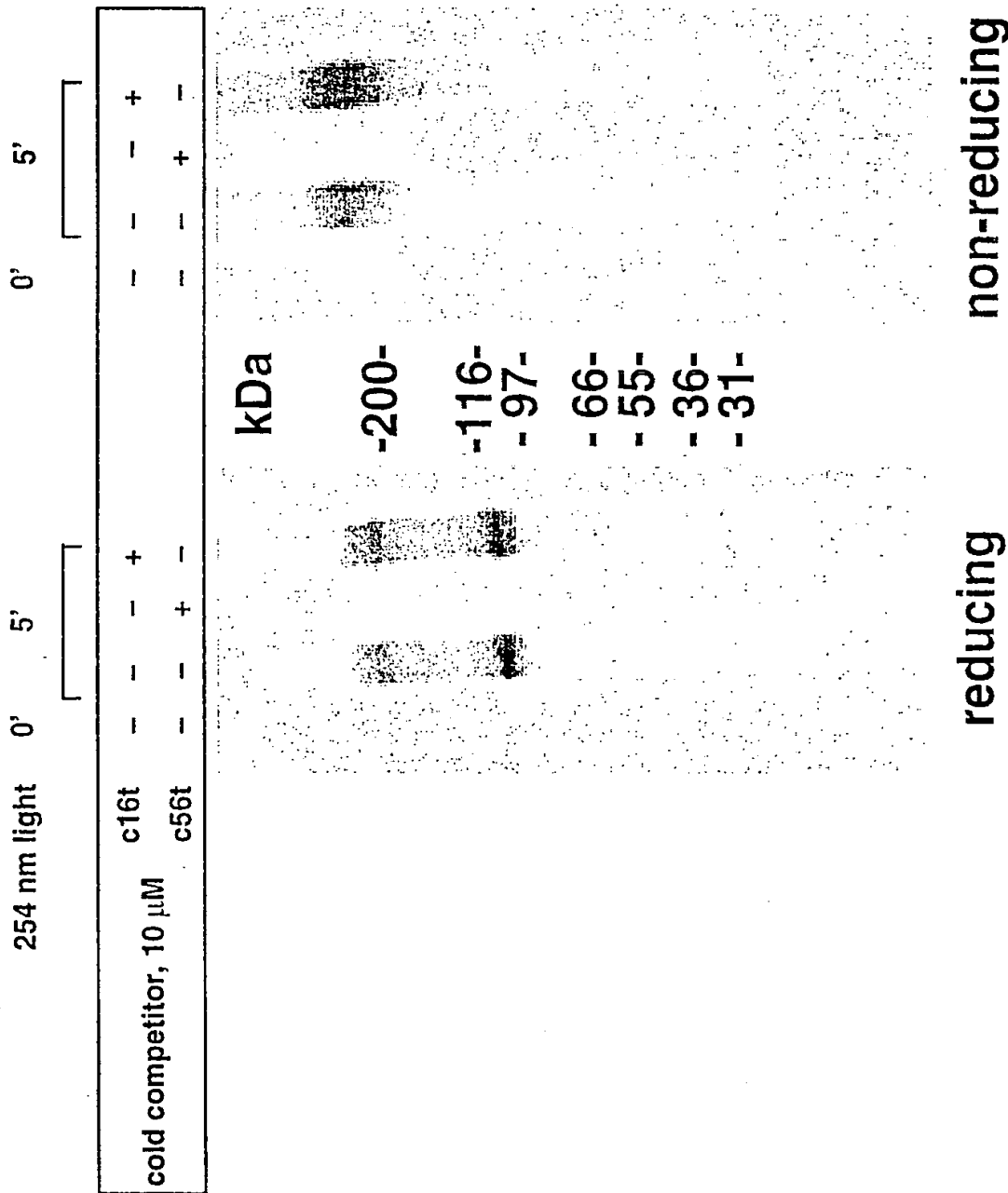


FIGURE 2

3/7

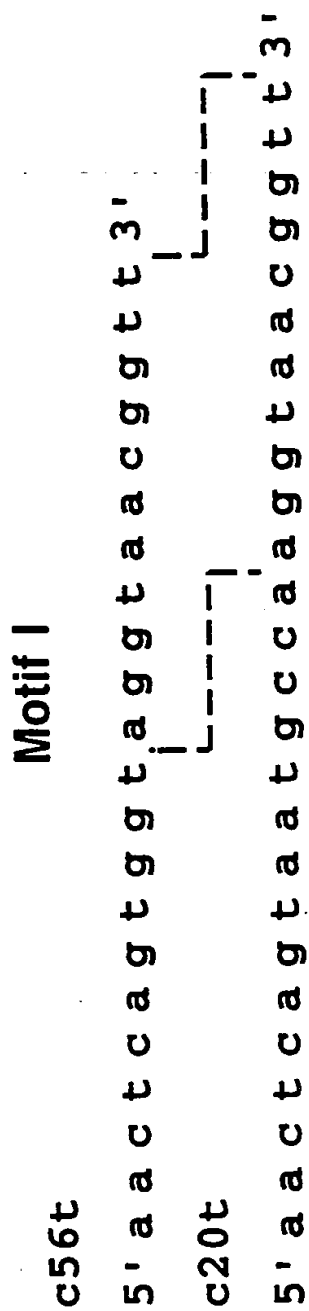


FIGURE 3A

Motif II

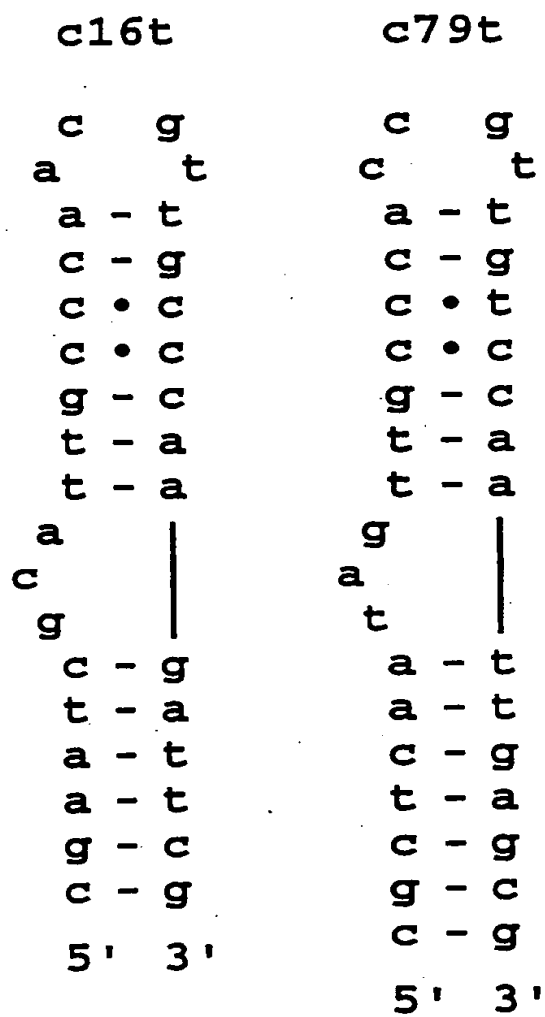


FIGURE 3B

5/7

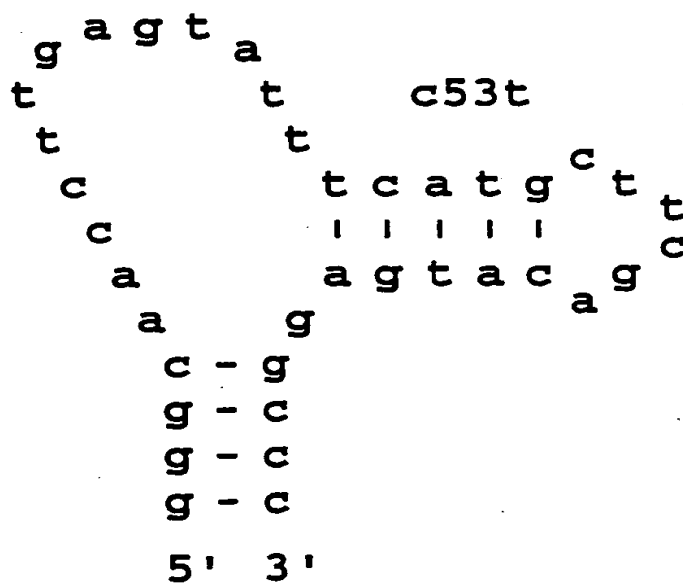
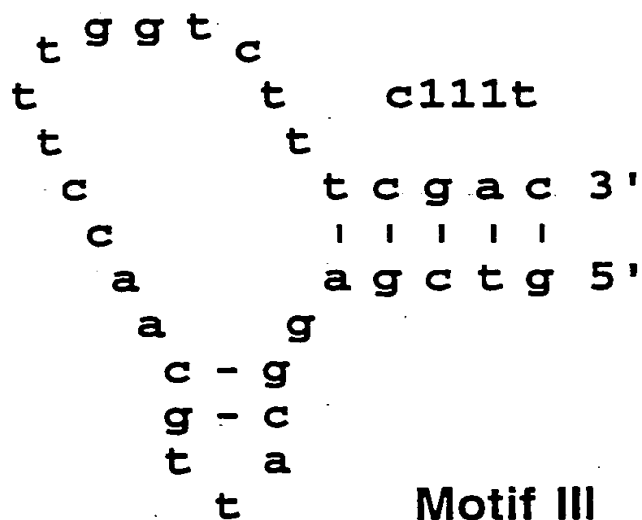


FIGURE 3C

6/7

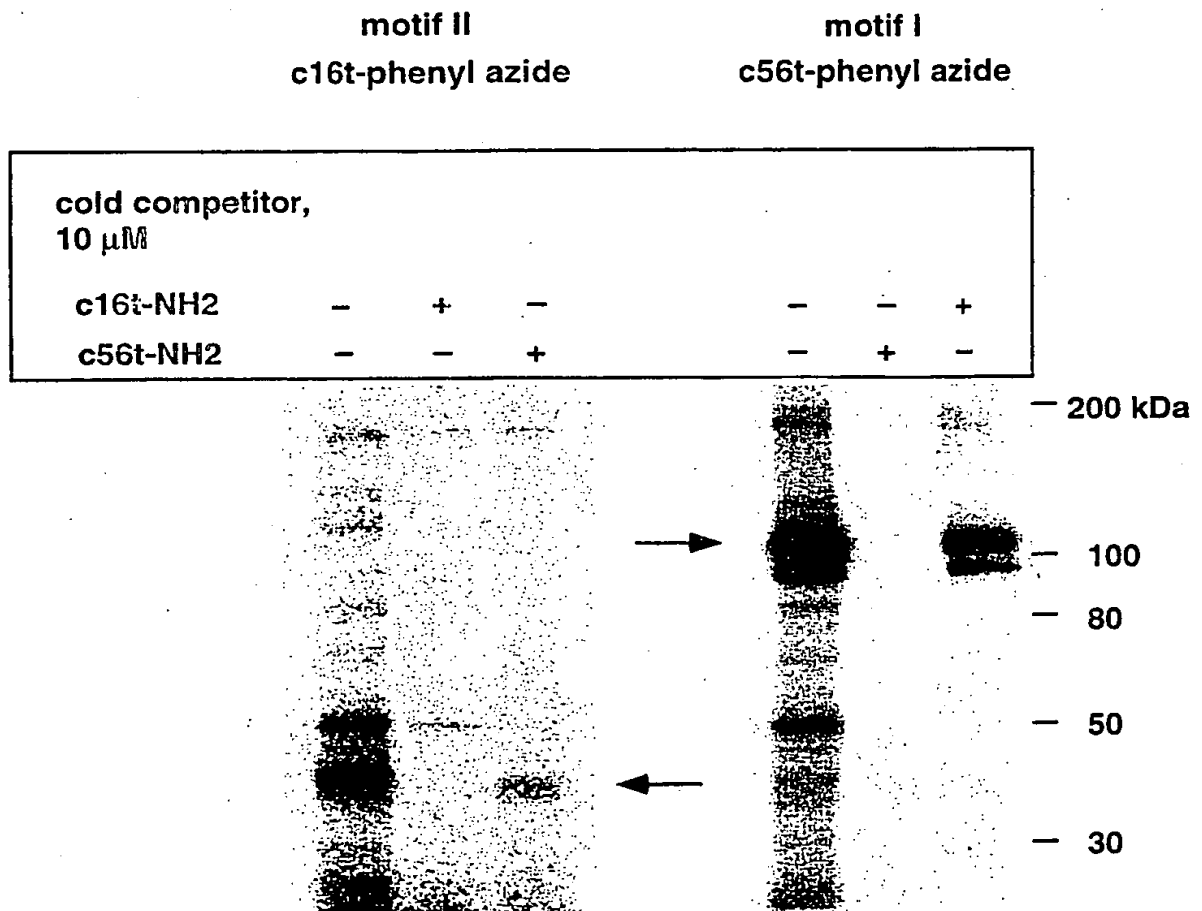


FIGURE 4

3 rounds photoaffinity crosslinking/ band isolation/ amplification

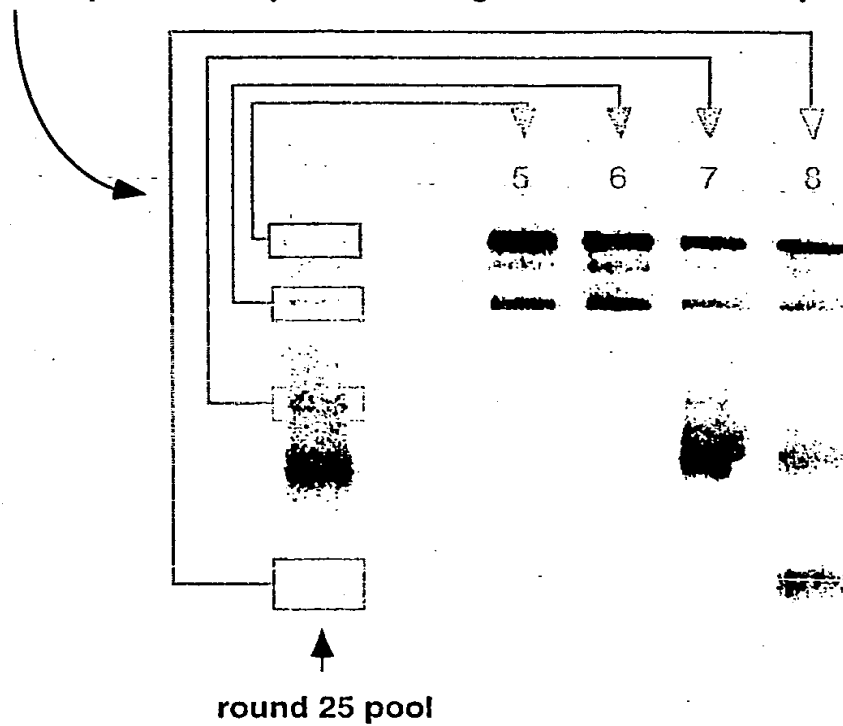


FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/06060

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/02, 21/04; C12P 19/34; C12Q 1/68

US CL : 435/6, 91.2; 536/22.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/22.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/14843 A1 (GILEAD SCIENCES, INC.) 03 SEPTEMBER 1992, PAGES 29-31, 94-102	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search	Date of mailing of the international search report
25 JULY 1996	29 AUG 1996

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